We are more than the sum of our genes

Klar 1998

Lecture 14: Epigenetics I

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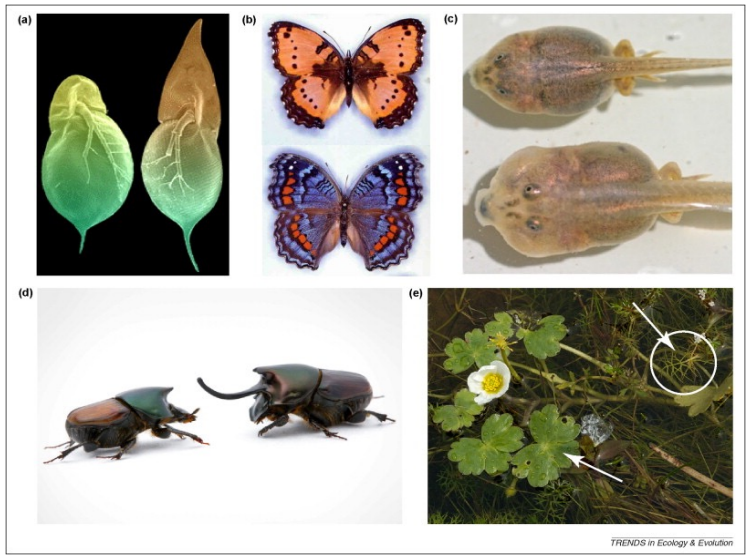
Defining epigenetics

Epigenetic phenomenon - a change in phenotype that is heritable but does not involve DNA mutation -

Biological examples of epigenetics phenotypes



X-inactivation

Defining epigenetics

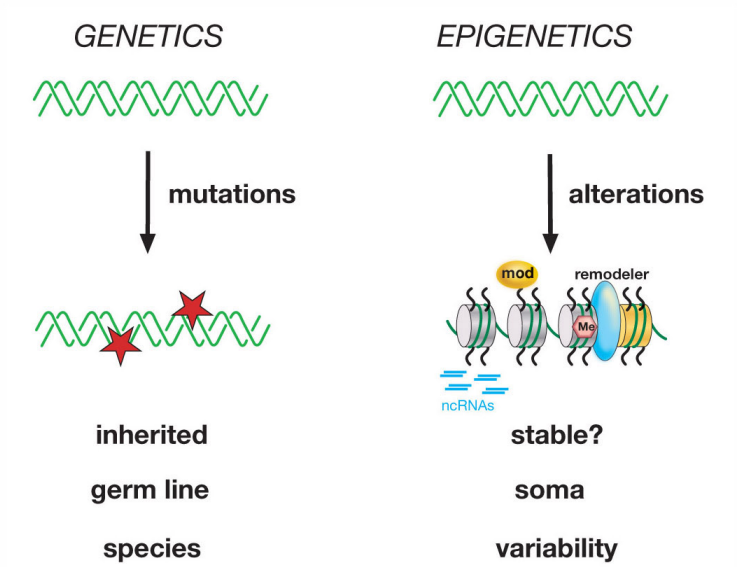


Figure 3. Genetics Versus Epigenetics

GENETICS: Mutations (*red stars*) of the DNA template (*green helix*) are heritable somatically and through the germ line. *EPIGENETICS:* Variations in chromatin structure modulate the use of the genome by (1) histone modifications (mod), (2) chromatin remodeling (*remodeler*), (3) histone variant composition (*yellow nucleosome*), (4) DNA methylation (*Me*), and (5) noncoding RNAs. Marks on the chromatin template may be heritable through cell division and collectively contribute to determining cellular phenotype.

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Epigenetics can be molecularly defined as:

"The sum of the alterations to the chromatin template that collectively establish and propagate different patterns of gene expression (transcription) and silencing from the same genome."

Post-translational histone modifications

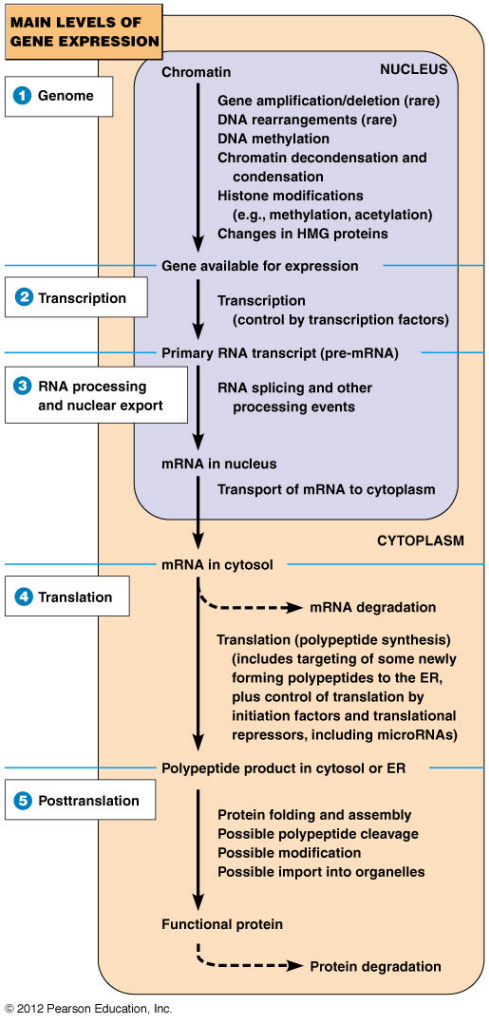
Energy-dependent chromatin-remodelling that mobilize or alter nucleosome structures

Dynamic shuffling of new histone variants in and out of nucleosomes

DNA methylation

Noncoding RNA

The chromatin state and how it affects gene expression


MAIN LEVELS OF GENE EXPRESSION
Chromatin
NUCLEUS
1 Genome
Gene amplification/deletion (rare)
DNA rearrangements (rare)
DNA methylation
Chromatin decondensation and condensation
Histone modifications (e.g., methylation, acetylation)
Changes in HMG proteins
Gene available for expression
2 Transcription
Transcription (control by transcription factors)
Primary RNA transcript (pre-mRNA)
3 RNA processing and nuclear export
RNA splicing and other processing events
mRNA in nucleus
Transport of mRNA to cytoplasm
CYTOPLASM
mRNA in cytosol
4 Translation
mRNA degradation
Translation (polypeptide synthesis) (includes targeting of some newly forming polypeptides to the ER, plus control of translation by initiation factors and translational repressors, including microRNAs)
Polypeptide product in cytosol or ER
5 Posttranslation
Protein folding and assembly
Possible polypeptide cleavage
Possible modification
Possible import into organelles
Functional protein
Protein degradation
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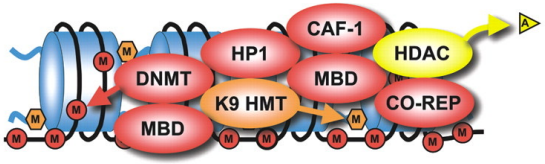
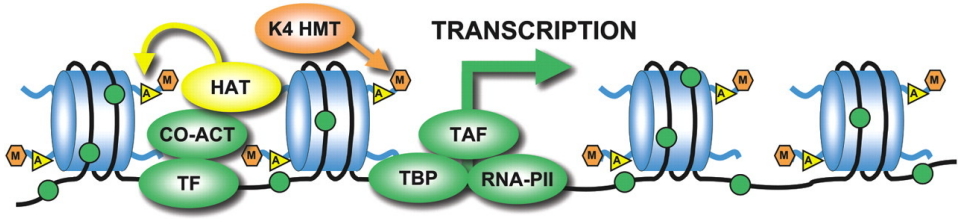
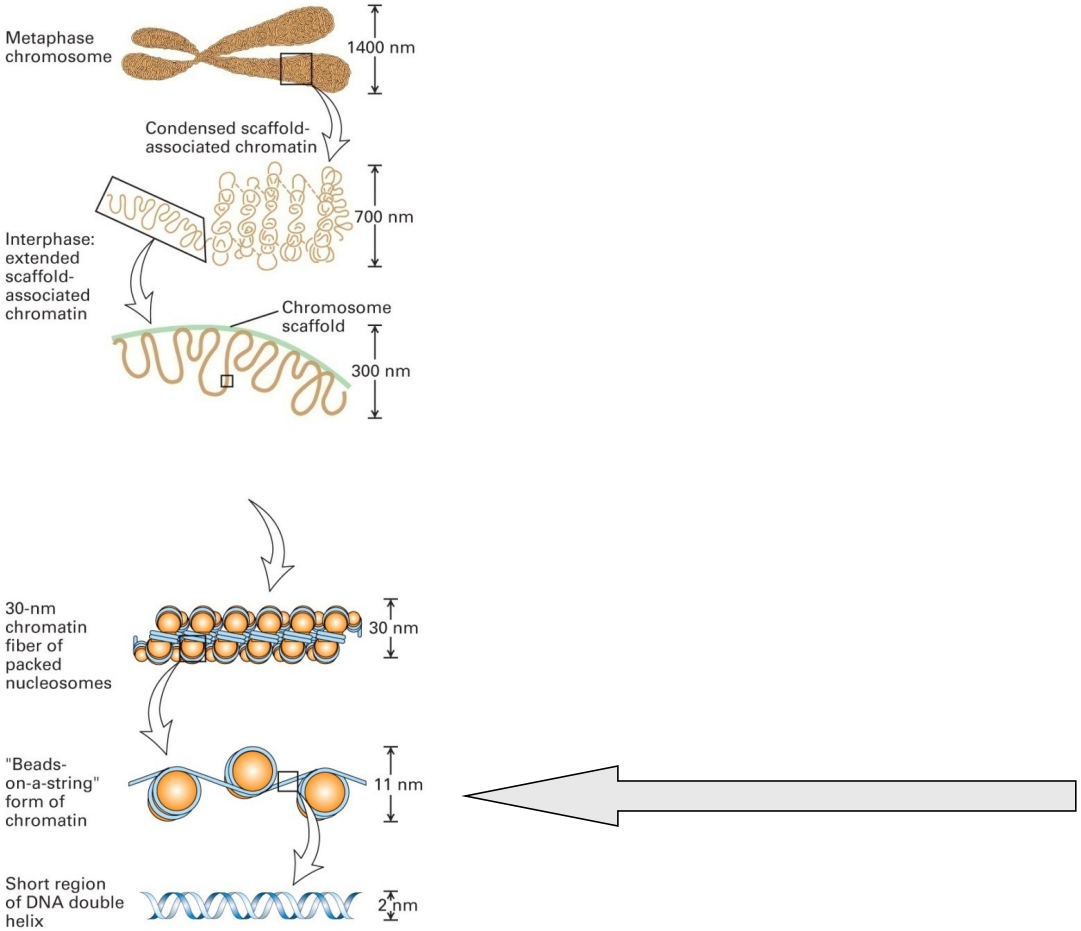
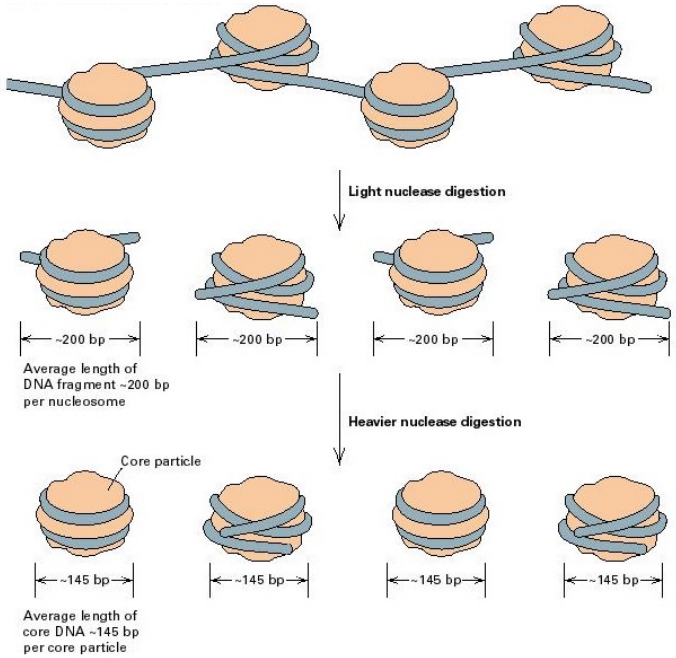




The chromatin template





The chromatin template

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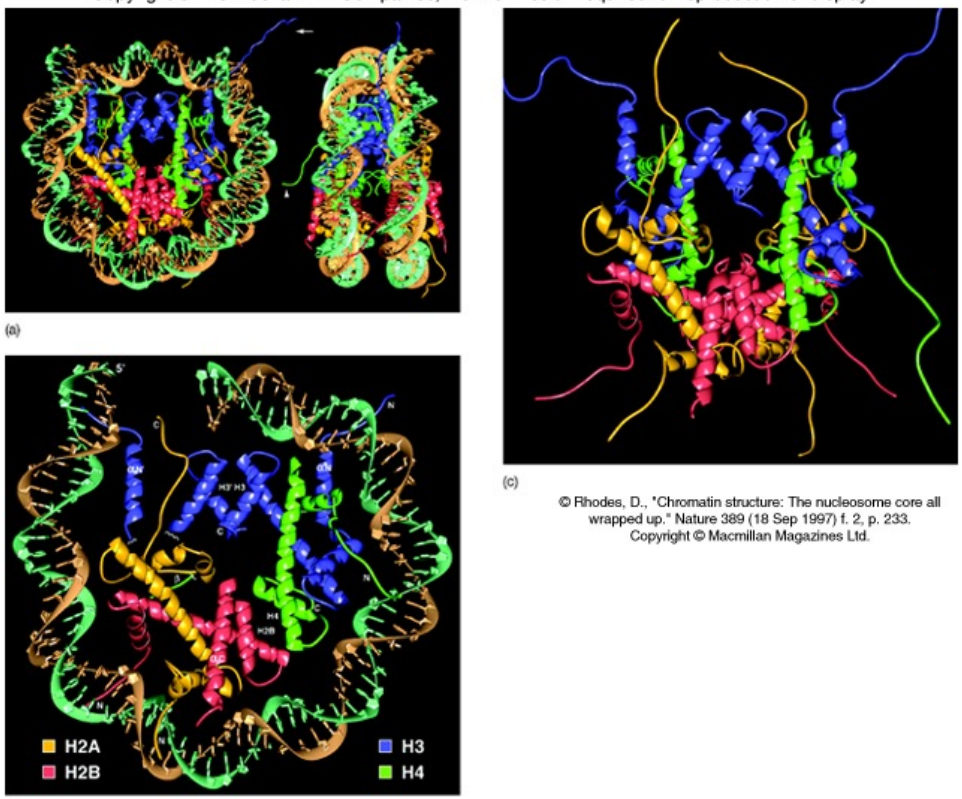

© Rhodes, D., "Chromatin structure: The nucleosome core all wrapped up." Nature 389 (18 Sep 1997) f. 2, p. 233. Copyright © Macmillan Magazines Ltd.

© Luger, K., A.W. Mader, R.K. Richmond, D.F. Sargent, and T. J. Richmond. "Crystal Structure of the Nucleosome Core Particle at 2.8A Resolution." Nature 389 (18 Sep 1997) f. 1, p. 252. Copyright © Macmillan Magazines Ltd.

NUCLEOSOMES

Protein octamer containing 2 molecules of each canonical (or core) histone (H2A, H2B, H3 and H4**)**

CORE HISTONES

Small and highly basic,

Globular domain and flexible “histone tails”, which protude from the surface of the nucleosome

The chromatin template

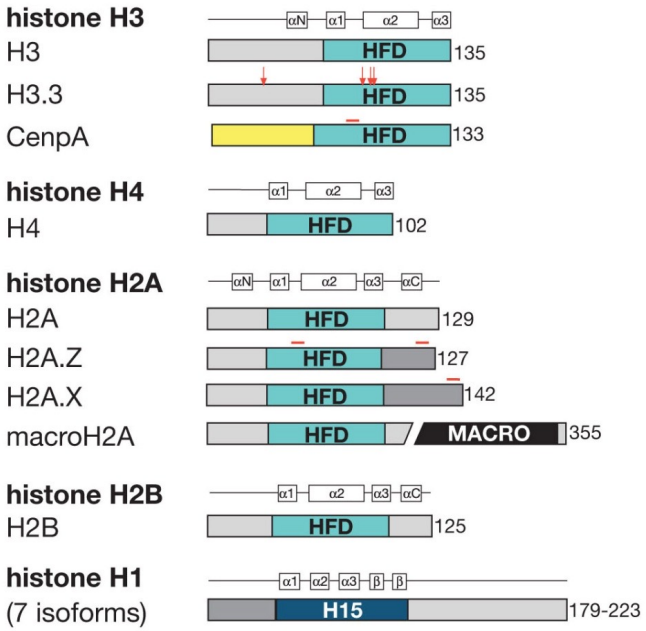

Figure 12. Histone Variants

Protein domain structure for the core histones (H3, H4, H2A, H2B), linker histone H1, and variants of histones H3 and H2A. The histone fold domain (HFD) where histone dimerization occurs, and regions of the protein that differ in histone variants (shown in *red*) are indicated.

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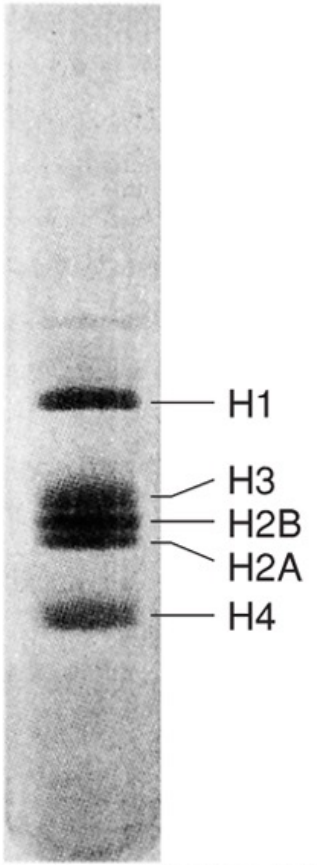

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The chromatin template

HISTONE TAILS - many residues are subject to extensively posttranslational modifications

Figure 6. Sites of Histone Tail Modifications

The amino-terminal tails of histones account for a quarter of the nucleosome mass. They host the vast majority of known covalent modification sites as illustrated. Modifications do also occur in the globular domain (*boxed*), some of which are indicated. In general, active marks include acetylation (*turquoise Ac flag*), arginine methylation (*yellow Me hexagon*), and some lysine methylation such as H3K4 and H3K36 (*green Me hexagon*). H3K79 in the globular domain has anti-silencing function. Repressive marks include H3K9, H3K27, and H4K20 (*red Me hexagon*), Green = active mark, red = repressive mark.

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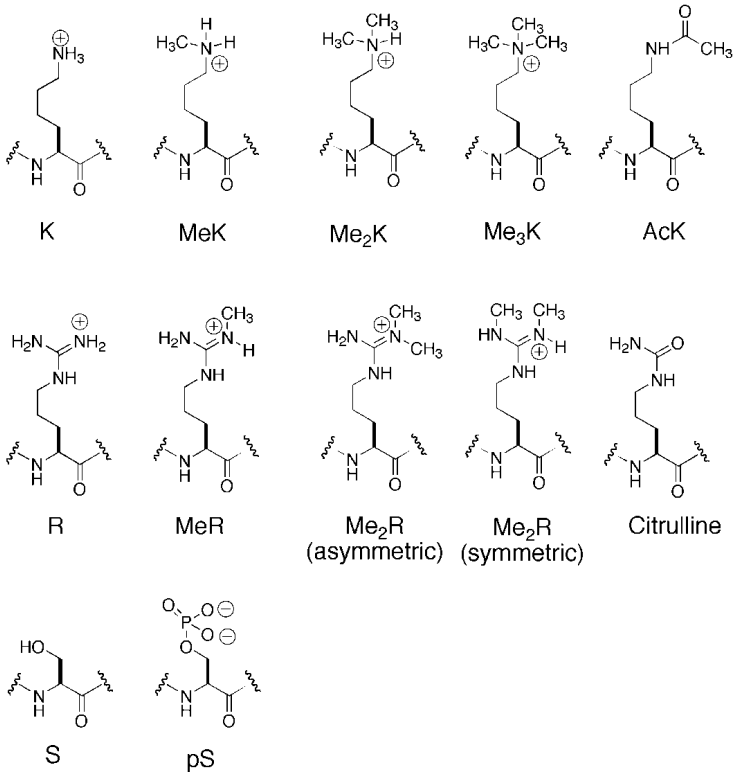
and more…. sumoylation, ADP-ribosylation, biotinylation, proline isomerization

Histone modifications and the histone code

Table 1 | Chromatin modifications

Mark*	Transcriptionally relevant sites†	Transcriptional role‡
DNA methylation		
Methylated cytosine (meC)	CpG islands	Repression
Histone PTMs		
Acetylated lysine (Kac)	H3 (9, 14, 18, 56), H4 (5, 8, 13, 16), H2A, H2B	Activation
Phosphorylated serine/threonine (S/Tph)	H3 (3, 10, 28), H2A, H2B	Activation
Methylated arginine (Rme)	H3 (17, 23), H4 (3)	Activation
Methylated lysine (Kme)	H3 (4, 36, 79) H3 (9, 27), H4 (20)	Activation Repression
Ubiquitylated lysine (Kub)	H2B (123§/120¶) H2A (119¶)	Activation Repression
Sumoylated lysine (Ksu)	H2B (6/7), H2A (126)	Repression
Isomerized proline (Pisom)	H3 (30–38)	Activation/repression

*The modification on either DNA or a histone.
†Well-characterized sites with regard to genomic location for DNA methylation or residues within histones for PTMs.
‡Whether the epigenetic mark is associated with activation or repression.
§Yeast (*Saccharomyces cerevisiae*).
¶Mammals.



Histone-modifying proteins

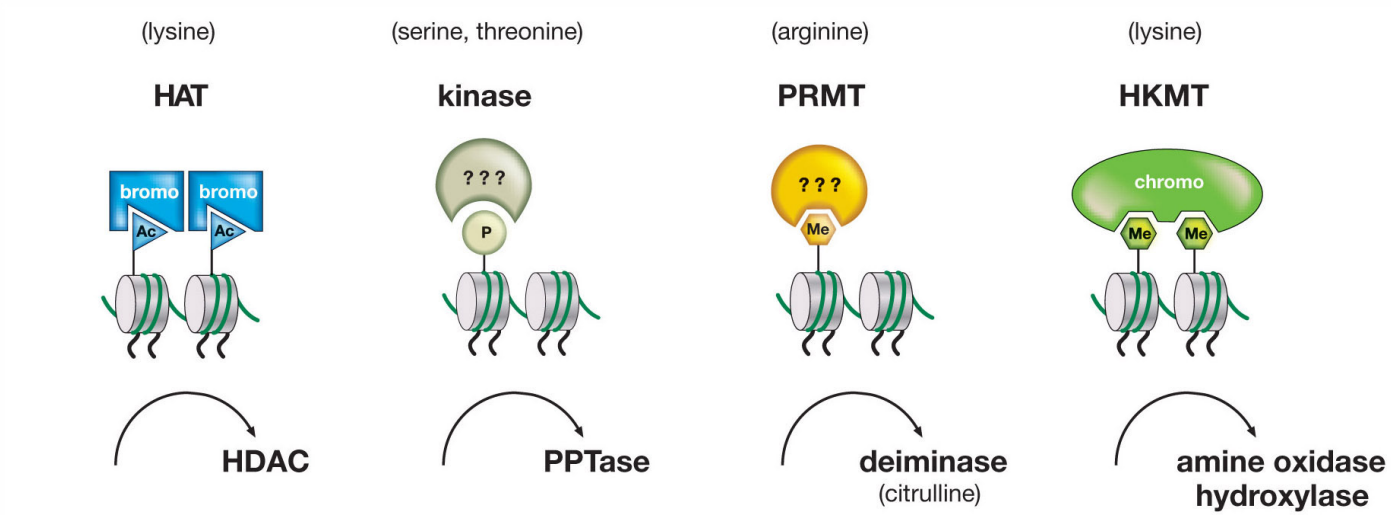


Figure 10. Histone-modifying Enzymes

Covalent histone modifications are transduced by histone-modifying enzymes ("writers") and removed by antagonizing activities. They are classified into families according to the type of enzymatic action (e.g., acetylation or phosphorylation). Protein domains with specific affinity for a histone tail modification are termed "readers." (HAT) Histone acetyltransferase; (PRMT) protein arginine methyltransferase; (HKMT) histone lysine methyltransferase; (HDAC) histone deacetylase; (PPTase) protein phosphatases; (Ac) acetylation; (P) phosphorylation; (Me) methylation.

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Histone modifications and the histone code

HOW CAN HISTONE MODIFICATIONS ALTER CHROMATIN STRUCTURE?

Change the **charge** of a residue to affect contact between histone-DNA, histone-histone, nucleosome-nucleosome.

Can form **binding surfaces** for a variety of histone modifying proteins

"The remarkable diversity and biological specificity associated with distinct patterns of covalent histone marks has caused us and others to favour the view that a histone `language' may be encoded on these tail domains that is read by other proteins or protein modules. We refer to this language as the `**histone code**'" *Strahl & Allis, 2000*

Histone modifications - acetylation

ACETYLATION – reduces the net positive charges of the lysine – unfold of chromatin

By chemically ligating modified tail peptides onto recombinant histone core preparations, it has been shown that **acetylation of H4K16 completely abolishes the ability of the tail domains to mediate nucleosome-nucleosome interactions**, which are required for chromatin condensation (*Shogren-Knaak et al., 2006, Science 311, 844-847).*

Hyperacetylated forms of H3 and H4 – localized preferentially in active genes exhibiting DNaseI sensitivity

DNaseI hypersensitive sites

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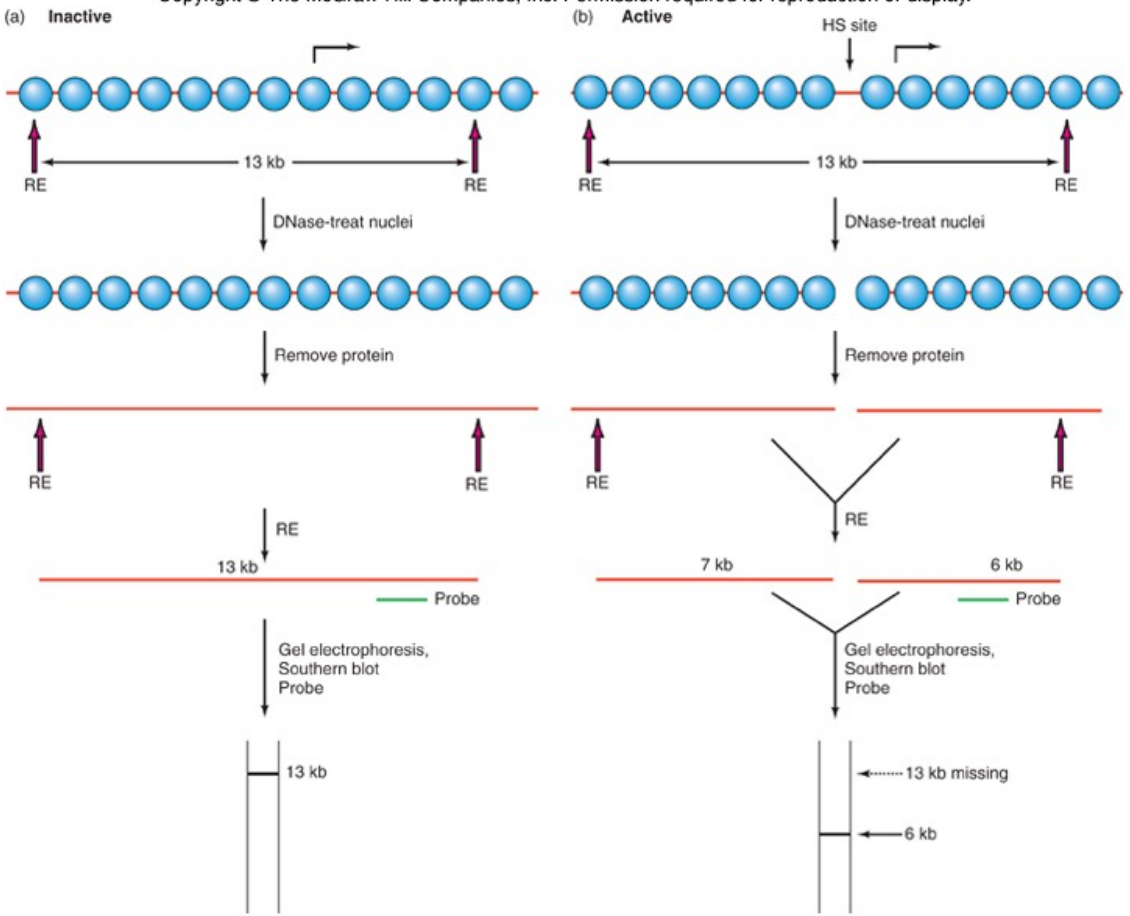
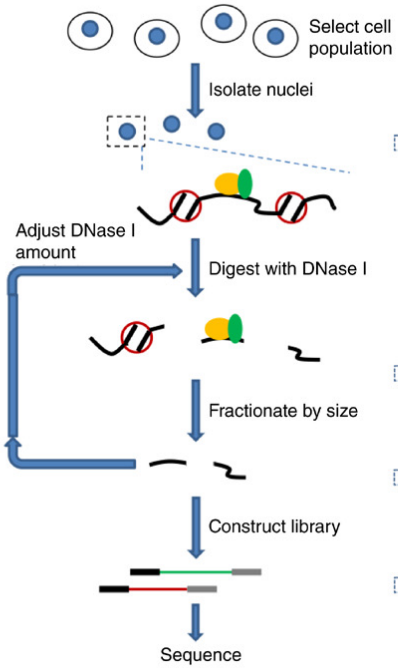
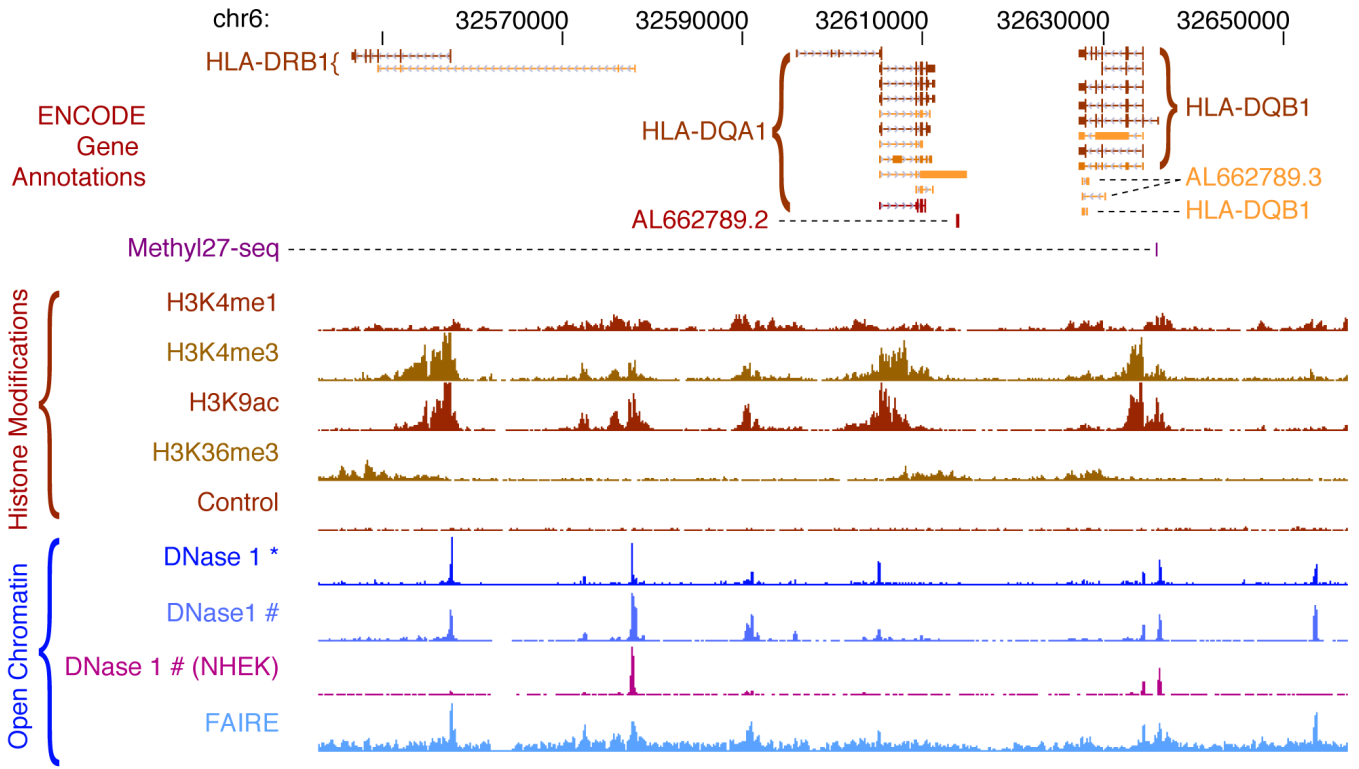


DHS landscape





Histone modifications - acetylation

ACETYLATION – reduces the net positive charges of the lysine – unfold of chromatin

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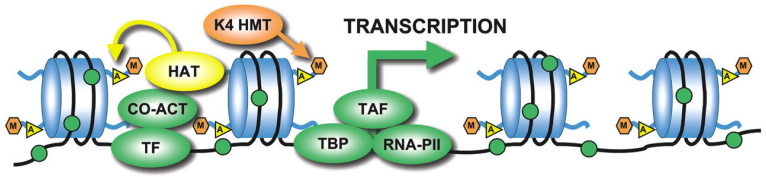
2. Hyperacetylated forms of H3 and H4 – localized preferentially in **active genes** exhibiting DNaseI sensitivity

3. Transcriptional **co-activators have histone acetyl-transferase (HAT) activity** (CBP/p300)



Histone modifications - acetylation

4. Treatment of cells with inhibitors of histone deacetylases (**HDACs**) has been shown to **increase transcription** of previously silent genes

5. The **reversal of acetylation** correlates with **transcriptional repression**

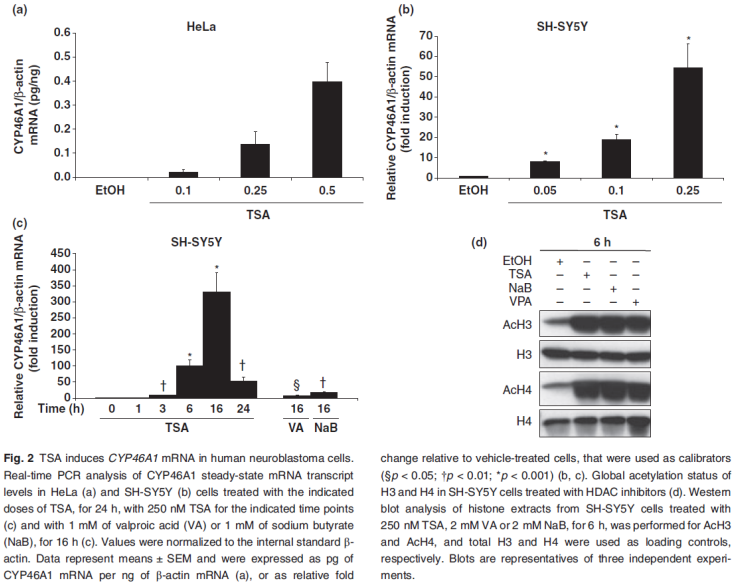
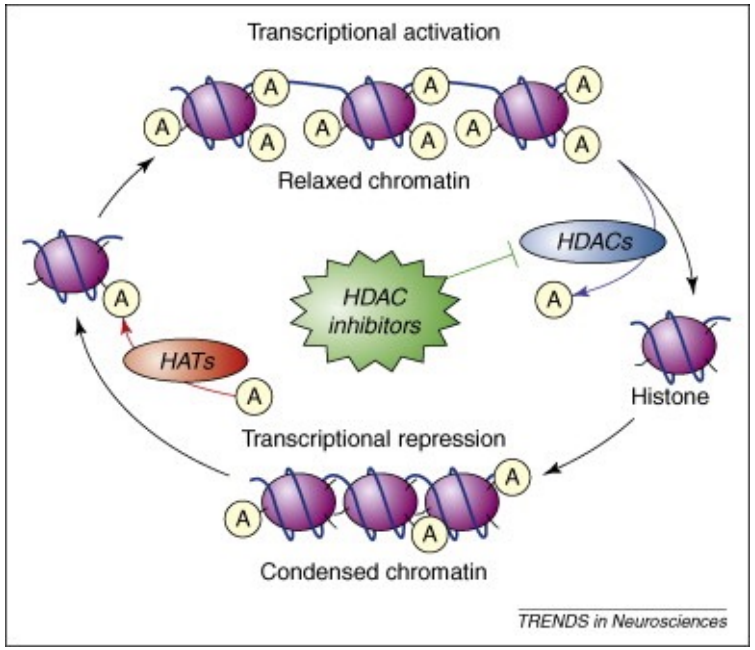
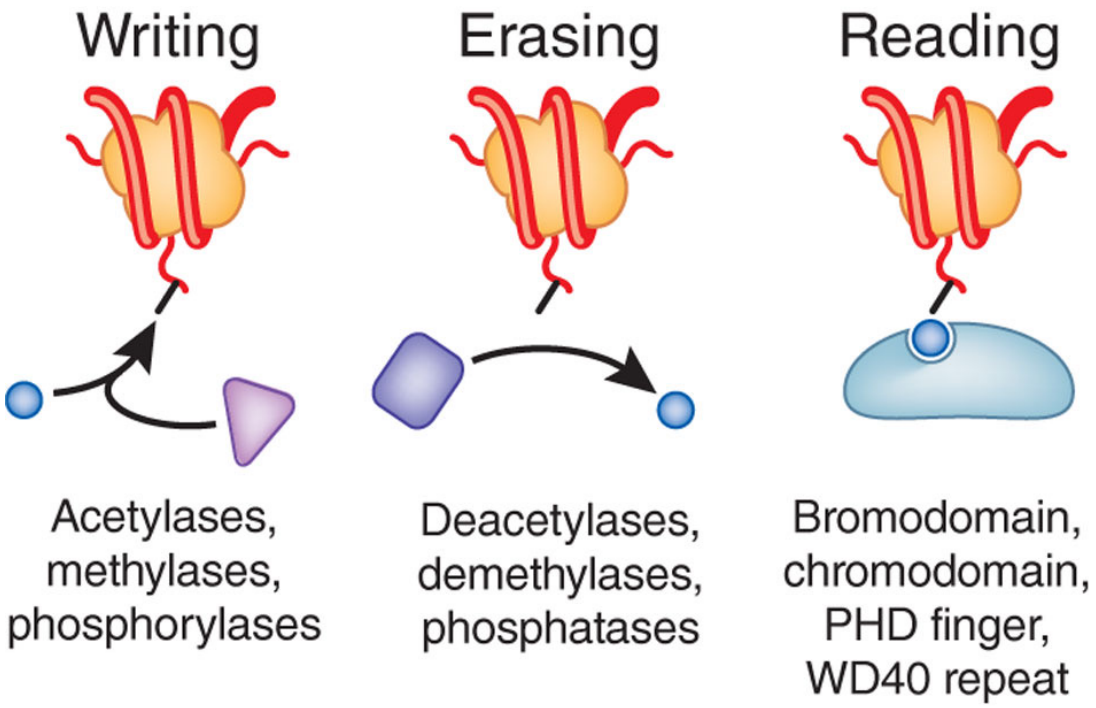


Fig. 2 TSA induces *CYP46A1* mRNA in human neuroblastoma cells. Real-time PCR analysis of CYP46A1 steady-state mRNA transcript levels in HeLa (a) and SH-SY5Y (b) cells treated with the indicated doses of TSA, for 24 h, with 250 nM TSA for the indicated time points (c) and with 1 mM of valproic acid (VA) or 1 mM of sodium butyrate (NaB), for 16 h (c). Values were normalized to the internal standard β-actin. Data represent means ± SEM and were expressed as pg of CYP46A1 mRNA per ng of β-actin mRNA (a), or as relative fold change relative to vehicle-treated cells, that were used as calibrators (§$p < 0.05$; †$p < 0.01$; *$p < 0.001$) (b, c). Global acetylation status of H3 and H4 in SH-SY5Y cells treated with HDAC inhibitors (d). Western blot analysis of histone extracts from SH-SY5Y cells treated with 250 nM TSA, 2 mM VA or 2 mM NaB, for 6 h, was performed for AcH3 and AcH4, and total H3 and H4 were used as loading controls, respectively. Blots are representatives of three independent experiments.



Histone-modifying/ interacting proteins



Histone-modifying/ interacting proteins

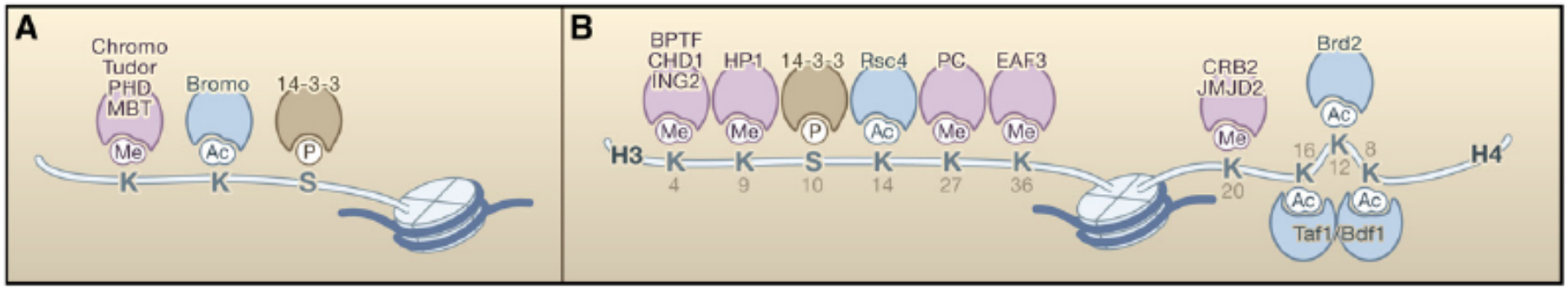


Figure 1. Recruitment of Proteins to Histones

(A) Domains used for the recognition of methylated lysines, acetylated lysines, or phosphorylated serines. (B) Proteins found that associate preferentially with modified versions of histone H3 and histone H4.

Context dependent histone modifications

Important histone modifications

You should now this!

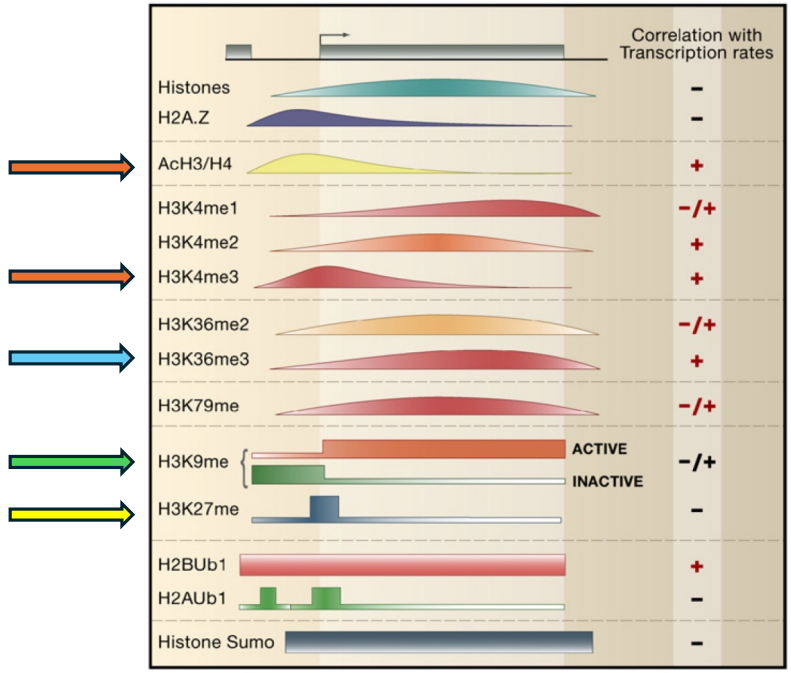
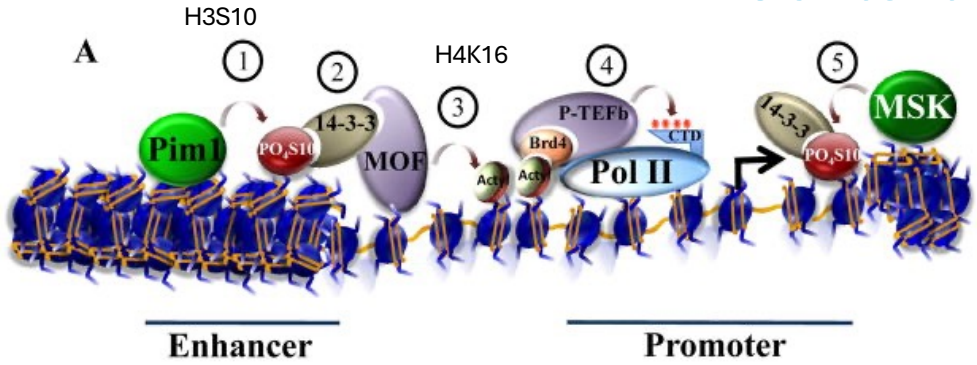


Figure 1. Genome-Wide Distribution Pattern of Histone Modifications from a Transcription Perspective

The distribution of histones and their modifications are mapped on an arbitrary gene relative to its promoter (5′ IGR), ORF, and 3′ IGR (original references were reviewed in Shilatifard, 2006; Workman, 2006). The curves represent the patterns that are determined via genome-wide approaches. The squares indicate that the data are based on only a few case studies. With the exception of the data on K9 and K27 methylation, most of the data are based on yeast genes.

Context dependent histone modifications

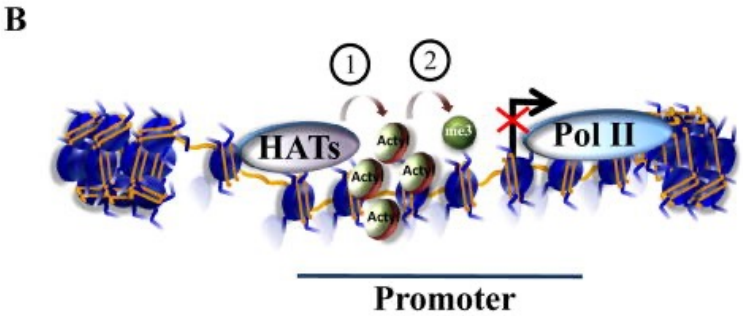


Transcriptional control of *FOS1L gene*

The timing, location, and perhaps identity of the H3 kinase, and not the H3S10 modification alone, determines downstream events

Correlations between histone acetylation and H3K4 methylation - does not lead to productive transcription

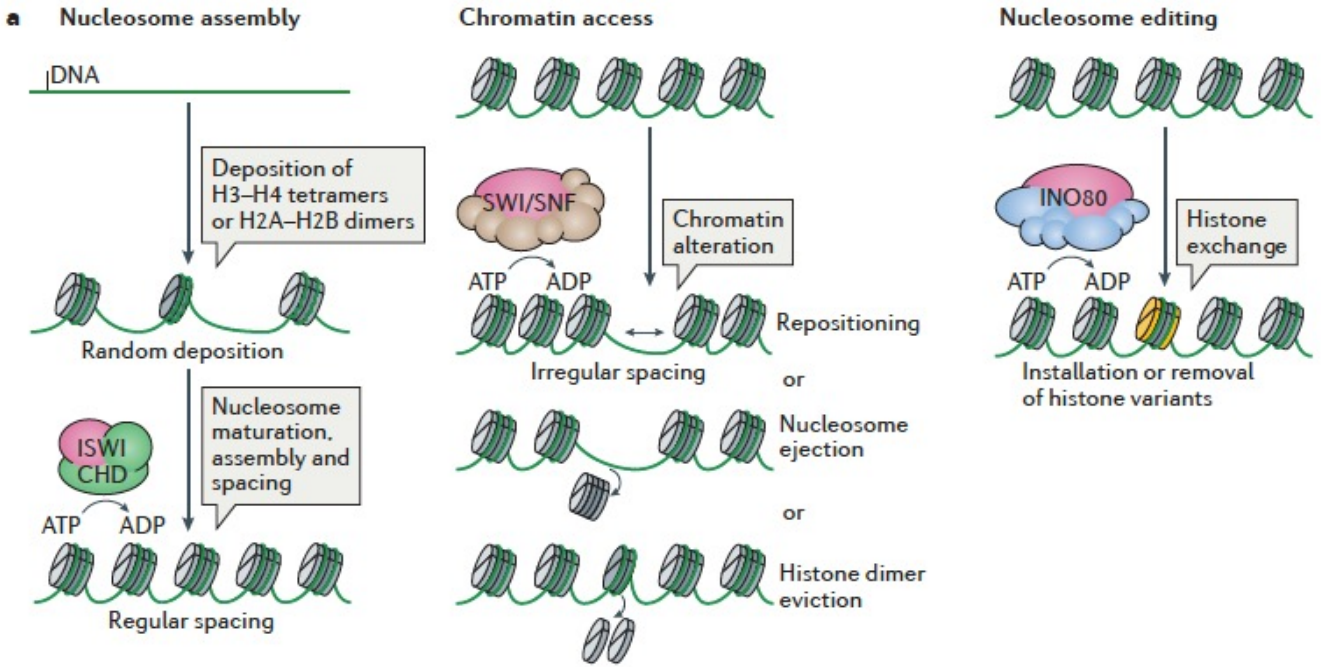
Distinct effects depending on the cellular context and upstream signaling events



Context dependent outcomes of histone crosstalk(A) Zippo and colleagues (Zippo et al., 2009) uncover a new form of histone crosstalk by studying the transcriptional control of *FOS1L*, a gene activated in response to serum. Activation requires the binding of PIM1 to the *FOS1L* enhancer. PIM1 is a kinase responsible for phosphorylation (P) of serine 10 on the histone H3 tail (H3S10). **Phosphorylated H3S10 creates a binding site for 14-3-3, a phosphoserine binding protein**. Acetylation (Ac) of lysine 16 on the H4 (H4K16) tail occurs through interaction of 14-3-3 with the histone acetyltransferase MOF. **Acetylated H4K16 can in turn form a binding site for the bromodomain-containing protein Brd4, a component of P-TEFb, a kinase that phosphorylates the C-terminal domain of RNA Pol II to facilitate transcription elongation**. However, at an earlier stage of serum stimulation, an MSK1/2 kinase is recruited to the promoter where it phosphorylates H3S10. 14-3-3 is then recruited to the promoter, but unlike the situation at the enhancer, MOF is not recruited to the promoter. **Thus, the timing, location, and perhaps identity of the H3 kinase, and not the H3S10 modification alone, determines downstream events.** **(B)** Another example of how the order of implementation of histone modifications can affect transcription comes from work from Wang et al. (2009). They report that despite correlations between histone acetylation and H3K4 methylation, artificially increasing acetylation through treatment of cells with deacetylase inhibitors (HDACs) does not lead to productive transcription, despite the presence of H3K4 methylation and Pol II recruitment. **Therefore, patterns of histone modifications cannot simply be "read", but have distinct effects depending on the cellular context and upstream signaling events.**

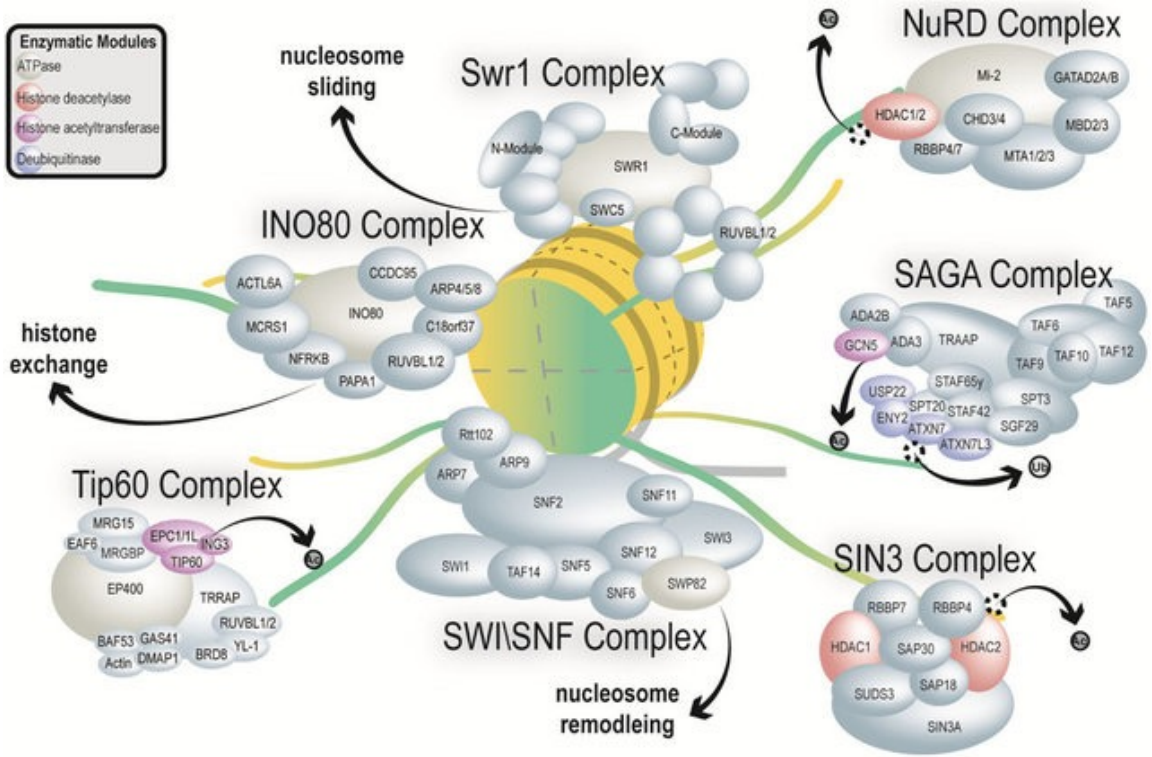
ATP-dependent chromatin remodelling

ATP- CHROMATIN-REMODELLING ENZYMES – utilize de energy of ATP hydrolysis to mobilize nucleosomes along DNA, evict histones off DNA or promote exchange of histone variants



ATP-dependent chromatin remodelling

The compositional diversity of remodellers enables specific interactions with particular transcription factors and/or histone modifications to specify targeting.



Ordered and cyclic recruitment of TFs and epigenetic writers

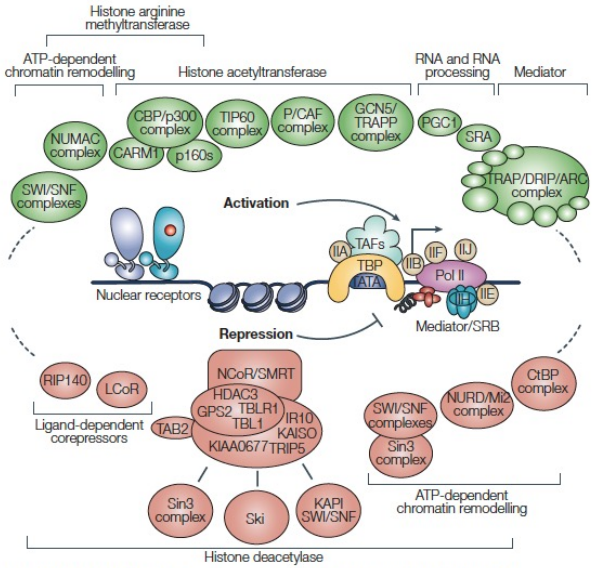


Figure 2 | **Coactivator and corepressor complexes are required for nuclear-receptor-mediated transcriptional regulation.** The regulation of a general transcription unit by nuclear receptors requires a vast number of coregulatory complexes that have various functions and enzymatic activities. Coactivator complexes (green) include factors that contain ATP-dependent chromatin remodelling activity[14,38,52], histone arginine methyltransferases[145–147], histone acetyltransferases[103,148,149], as well as factors that are involved in RNA processing[20,150] and components of the so-called Mediator complex that mediate the interaction with the RNA polymerase II (pol II) machinery[151–154]. Conversely, corepressors (red) include ATP-dependent chromatin remodelling complexes[21,155–159], basal corepressors, such as NCoR and SMRT, which function as platforms for the recruitment of several subcomplexes that often contain histone deacetylase activity[13,27,88,122,123], and specific corepressors, such as LCoR and RIP140, which are surprisingly able to recruit general corepressors on ligand induction[10]. This schematic representation is useful to underline the numerous regulatory complexes that are involved in nuclear-receptor-mediated regulation; however, it is important to keep in mind that it does not illustrate the dynamics of their recruitment to the regulated transcription unit (see FIG. 3). IIA, IIB, IIE, IIF, IIH, IIJ, general transcription factors A, B, E, F, H, J; HDAC, histone deacetylase; LCoR, ligand-dependent nuclear-receptor corepressor; NCoR, nuclear-receptor corepressor; RIP140, receptor-interacting protein-140; SMRT, silencing mediator of retinoic acid and thyroid hormone receptors; TAF, TBP-associated factor; TBP, TATA-binding protein.

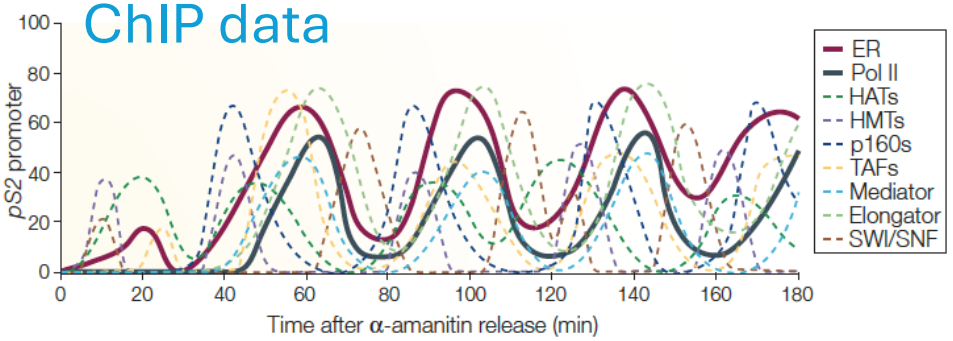


Figure 3 | **Ordered cofactor recruitment by the ER.** The diagram summarizes the observations of Metivier and colleagues on the kinetics of cofactor recruitment to the *pS2* promoter. In this kinetic ChIP analysis, the *pS2* promoter was stimulated with 10 nM oestradiol, and transcription by RNA polymerase II (pol II) was inhibited by treatment with α-amanitin[16]. For purposes of clarity, different factors that have the same enzymatic activities have been grouped together. However, this simplification might hide differences among proteins that belong to the same enzymatic class and that are represented as a single unit. For example, the histone acetyltransferase (HAT) GCN5 is always recruited slightly after the other family members, shown by the green curve. Also, in the first cycle, only p300, the HAT TIP60 and the histone methyltransferase (HMT) PRMT1 are engaged, whereas the other HATs and HMTs are not yet recruited. However, it is unclear whether this initial, non-productive cycle exists under physiological conditions, as the use of α-amanitin, although very important to distinguish the kinetics of ER-dependent recruitment from other basal activation events of the promoter, creates an artificial situation in which transcription has been completely shut off. The *y* axis represents the amount of immunoprecipitated *pS2* promoter expressed as a percentage of the input[16]. p160 proteins belong to the NCoA family of coactivators, components of the Mediator complex mediate pol II transcription, elongator factors are involved in transcription elongation and SWI/SNF are ATP-dependent chromatin remodelling factors. TAFs, TBP-associated factors.

We are more than the sum of our genes

Klar 1998

Lecture 15: Epigenetics II

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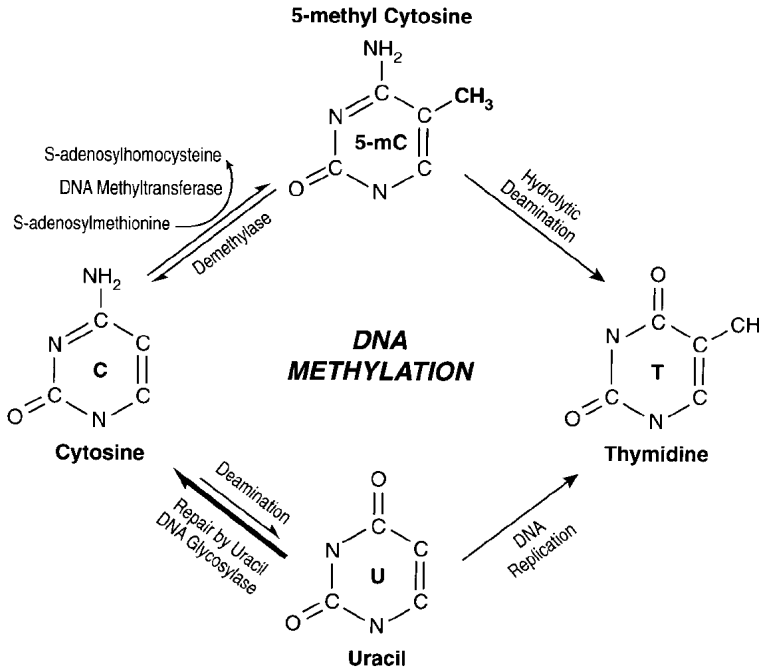
DNA methylation



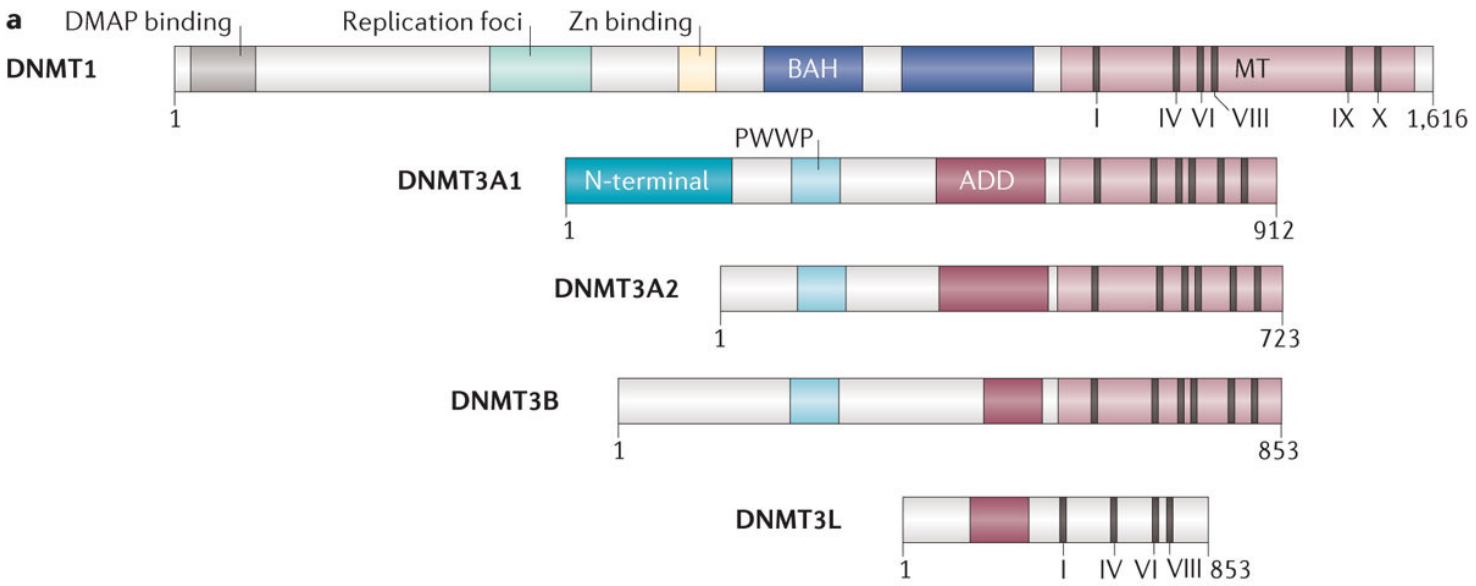
DNA METHYLATION in eukaryotes involves addition of a methyl group to the cytosine ring

In human somatic cells **5mC accounts for 1% of total DNA bases** and therefore affects 70-80% of all CpG dinucleotides in the genome

Nevertheless, eukaryotic genomes are **not uniformly methylated**, but contain methylated regions interspread with unmethylated domains

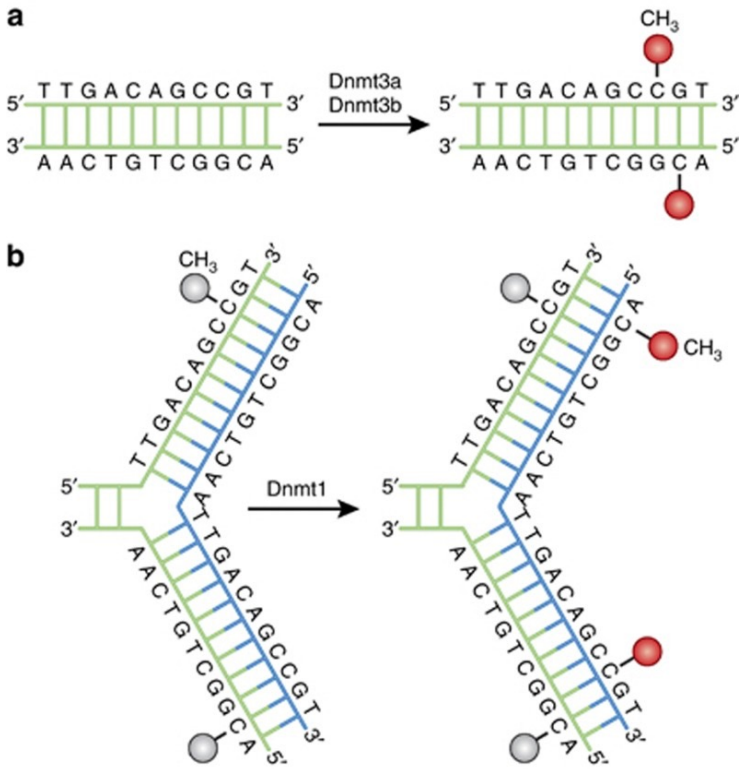
DNA methylation – the epigenetics writers

DNA methyltransferases



DNA methylation – the epigenetics writers

DNA methyltransferases



DNA methylation – the epigenetics writers

DNA methyltransferases

Table 1. Lessons from DNMT knockout experiments

Gene	Phenotype	Refs
DNMT1$^{-/-}$	Mutant ES cells viable and contain low levels of methylation and methyltransferase activity. Existence of another DNMT speculated. Recessive lethal phenotype in mice, embryos stunted with delayed development and reduced methylation levels	22,32
DNMT3a$^{-/-}$	Mice normal at birth but became runted and died at 4 weeks. Embryonic defects similar to those presented for DNMT1 mutant mice,[32] defective de novo methylation	29
DNMT3b$^{-/-}$	No mice born, embryos reveal many developmental defects including growth and neural tube defects, defective de novo methylation, DNMT3b mutations are responsible for ICF Syndrome	29
DNMT3L$^{-/-}$	Spermatogenesis, maternal methylation and genomic imprinting defects	34,35

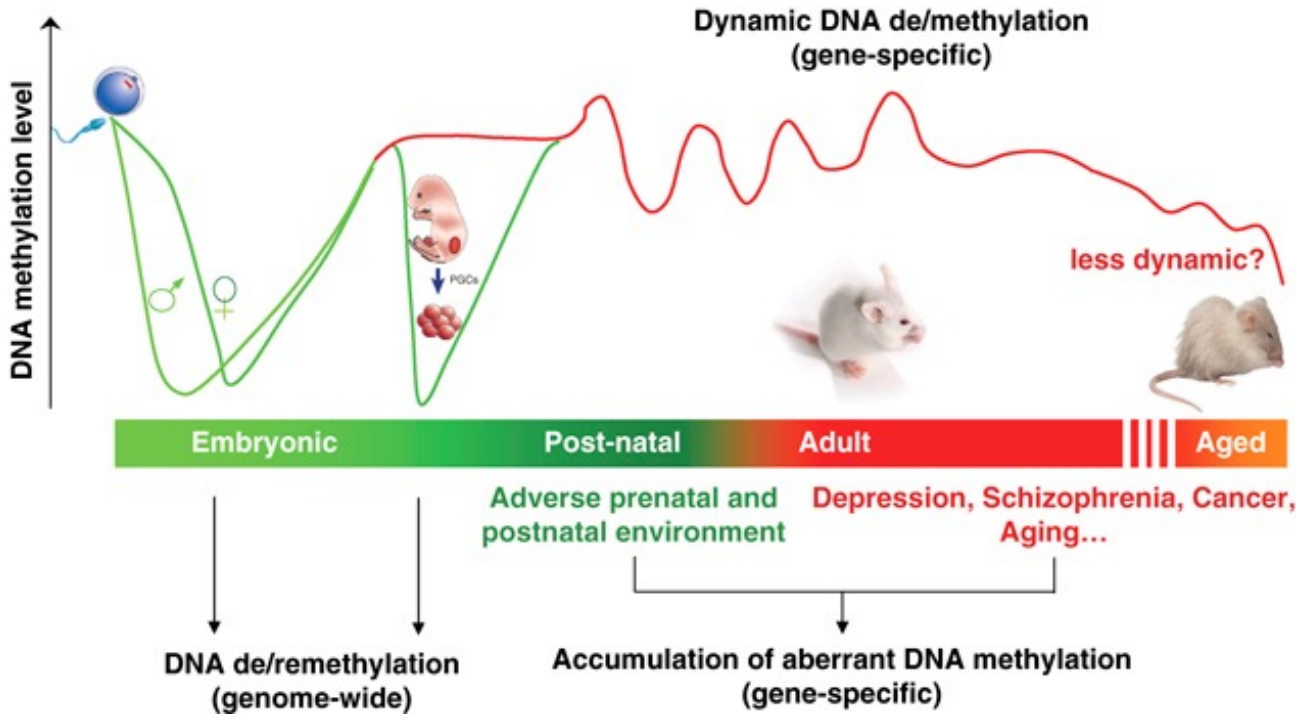
Table 2. DNMT-associated binding partners that modify chromatin

Binding partner	Binding region	HDAC activity	Proposed function	Refs
DNMT1				
HDAC1	RD	Yes	Chromatin remodeling, transcriptional silencing	36
HDAC2	RD	Yes	Chromatin remodeling, transcriptional silencing	37
DMAP1	RD	Yes	Histone deacetylation following DNA replication, transcriptional silencing	37
pRB	RD	Yes	Chromatin remodeling, transcriptional silencing	38
MBD3	RD	—	Binds hemi-methylated DNA, transcriptional silencing	39
PCNA	RD	—	Targeting to replication foci, maintain DNA methylation	21,40
DNMT3a/DNMT3b				
RP58	PHD	Yes	Maintain transcriptionally repressive chromatin in late S-phase	41,42
DNMT3L				
HDAC1	PHD	Yes	Transcriptional silencing	43,44

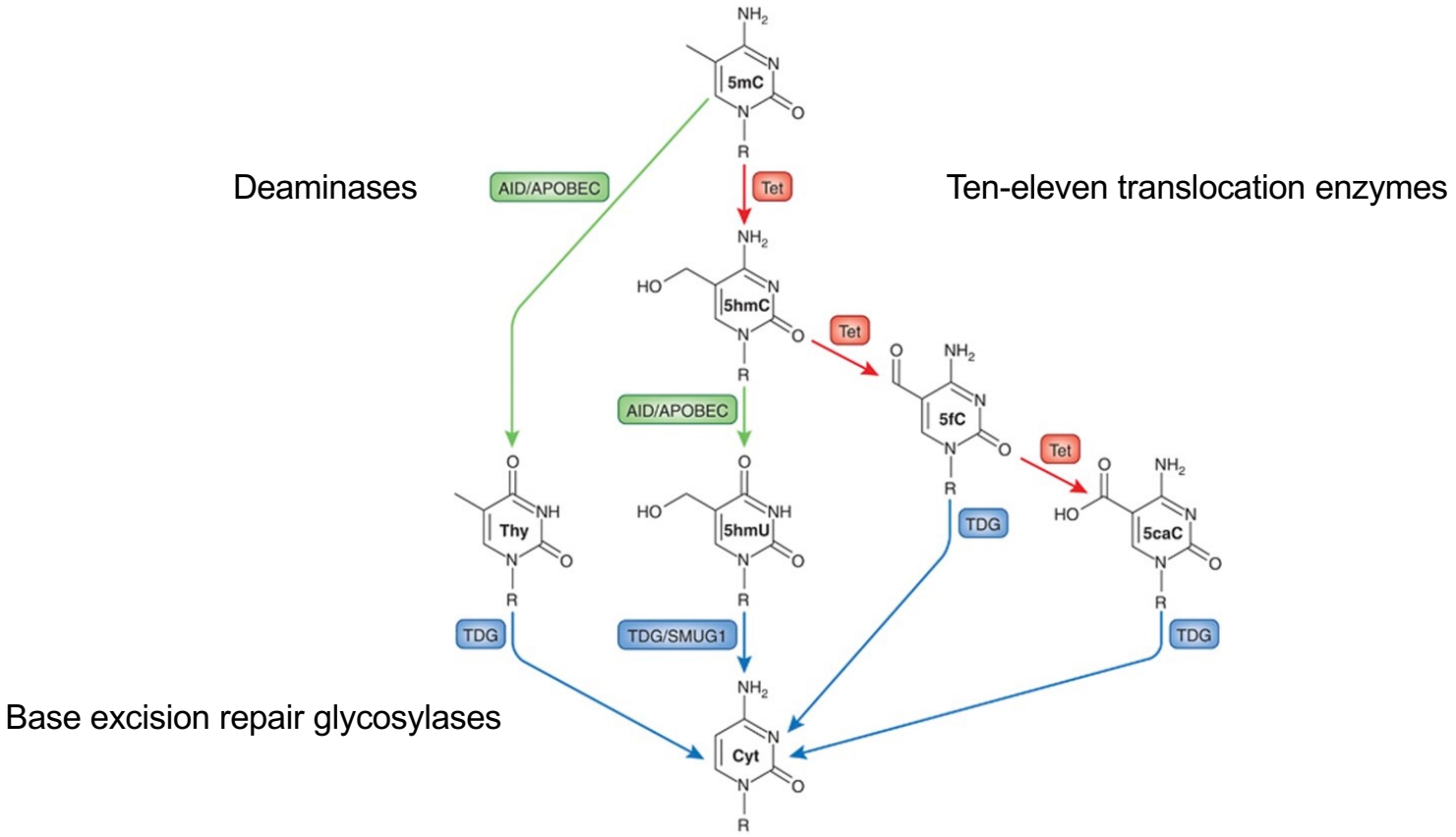
RD; N-terminal regulatory domain of DNMT1, PHD; ATRX-like PHD domain of DNMT3.

Passive DNA demethylation

Reduction in the DNMT levels gradually and passively removes DNA methylation in early development



Active DNA demethylation



DNA methylation

CpG ISLANDS – unmethylated GC-rich regions that possess high relative densities of CpG and are positioned at the 5' ends of genes

- earlier studies estimated that **~60% of human genes are associated with CpG islands**, of which the great majority are unmethylated at all stages of development and in all tissue types

- because many CpG islands are located at genes that have a tissue-restricted expression pattern, it follows that **CpG islands can remain methylation-free even when their associated gene is silent**

- a small but significant proportion of all **CpG islands become methylated during development, and when this happens the associated promoter is stably silent** – genomic imprinting and X chromosome inactivation

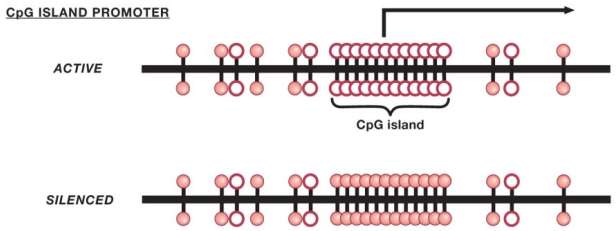


Figure 4. CpG Islands

CpG islands are regions of high CpG density that lack CpG methylation found at promoters of most human genes. Long-term silencing of the gene can be ensured by methylation of the CpG island region. For example, genes on the inactive X chromosome and certain imprinted genes are silenced in this way. Additionally, in cancer cells, certain genes are aberrantly silenced by CpG island methylation.

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de novo methylation:

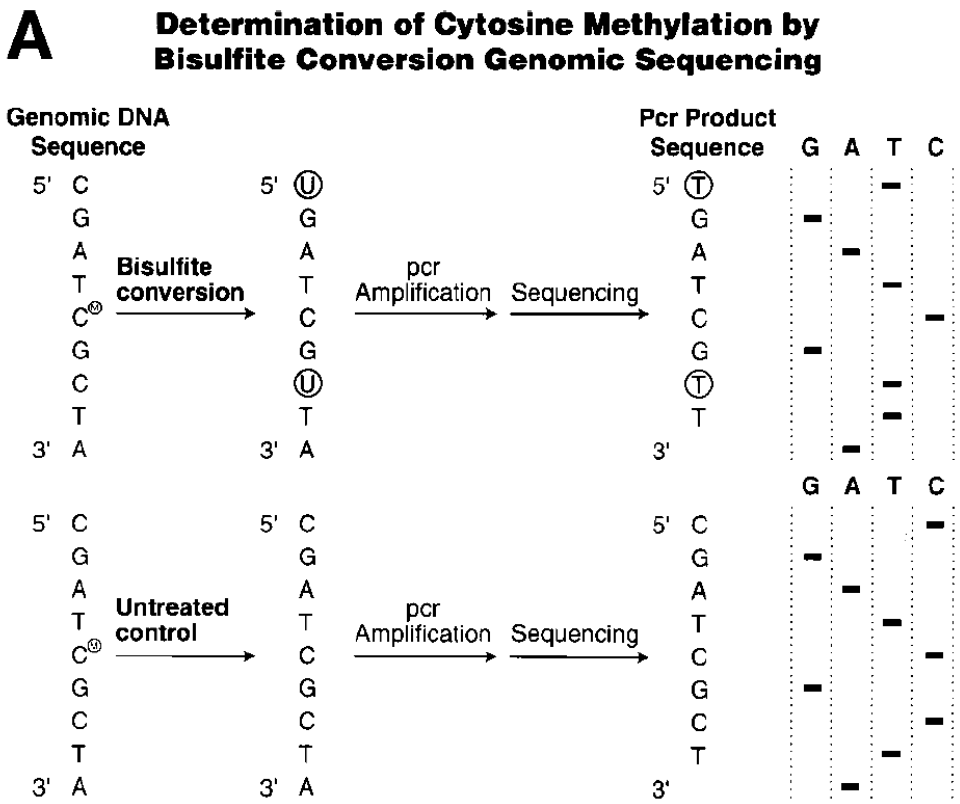
early embryo

aging

cancer

Methods to study DNA methylation

Gold standard approach – **bisulfite sequencing** - DNA is treated with sodium bisulfite which converts unmethylated cytosines to uracils



Methods to study DNA methylation

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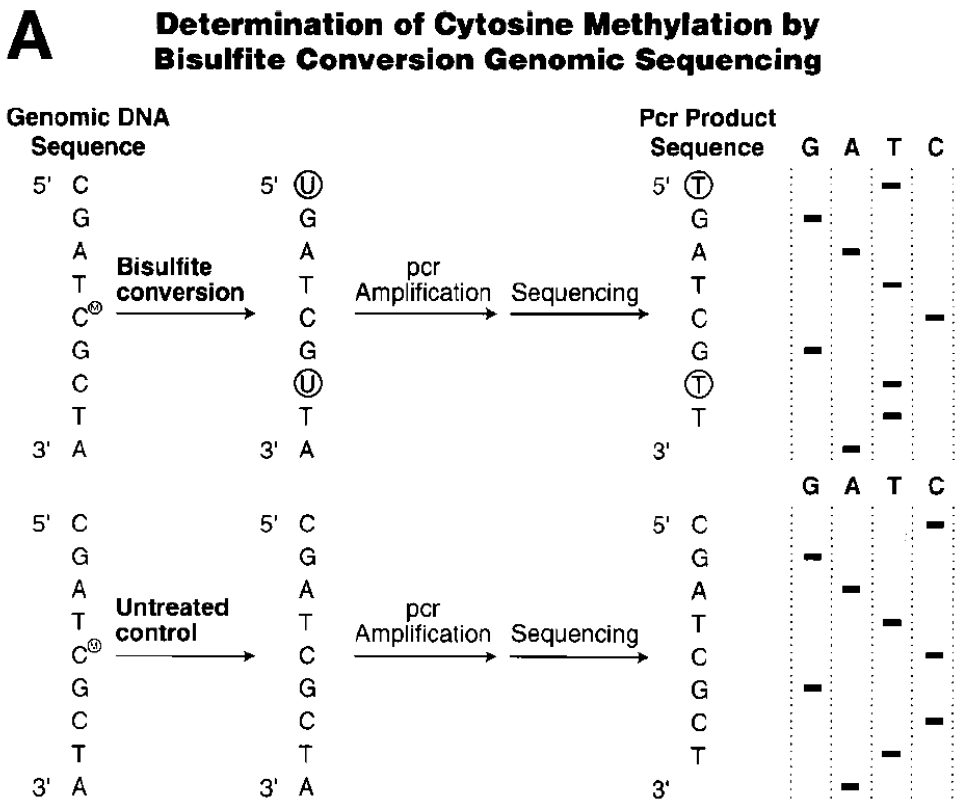
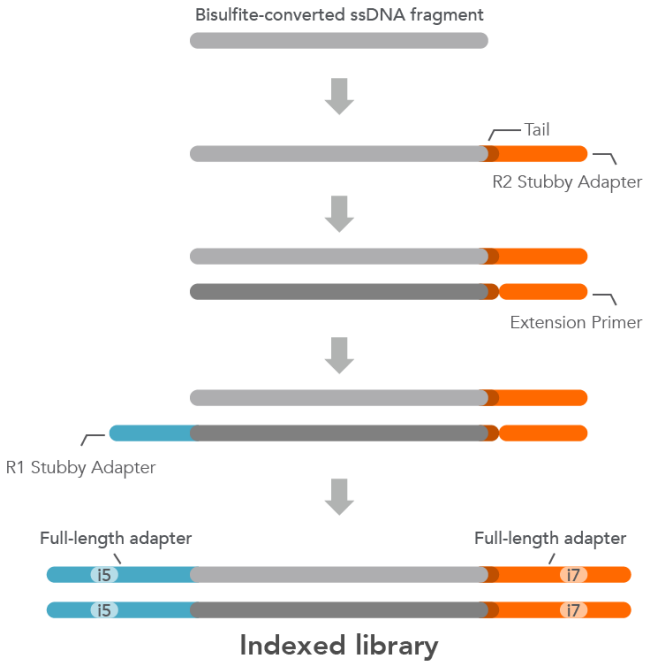
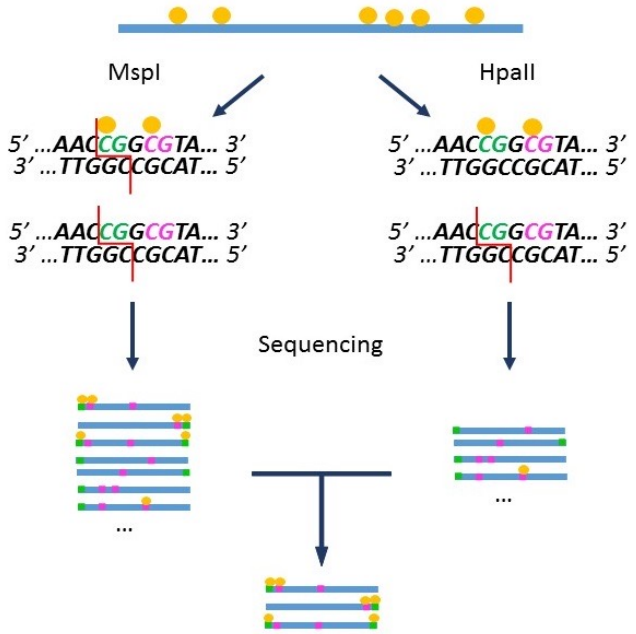
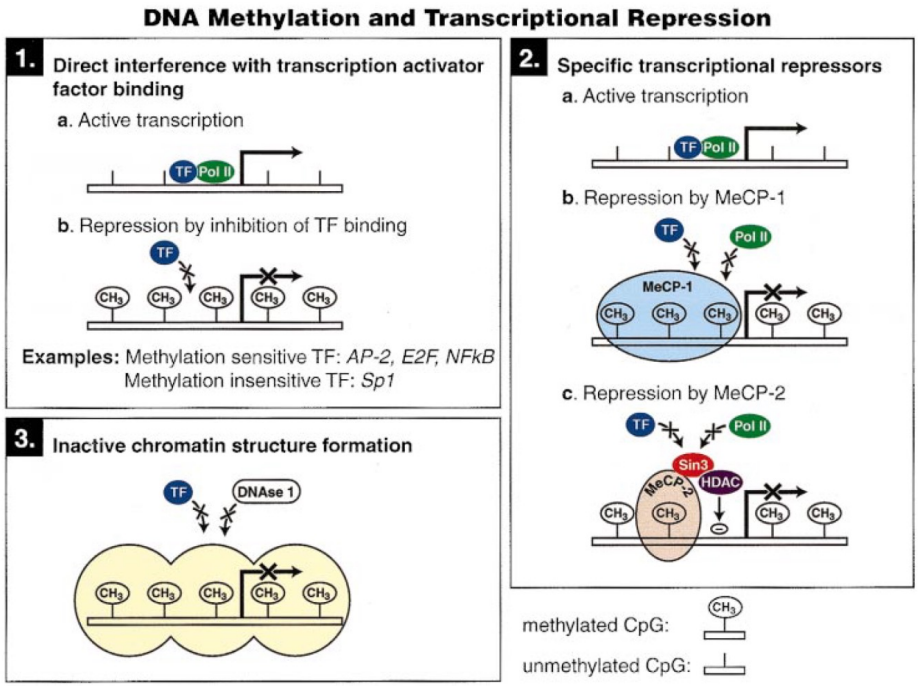


Whole genome Methyl-sequencing (Methyl-seq)

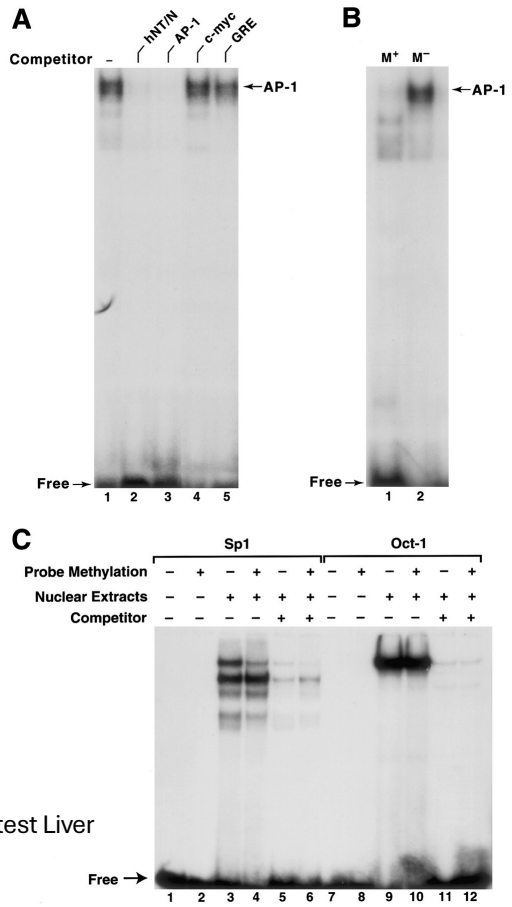




Mechanisms of DNA-methylation-mediated transcriptional repression

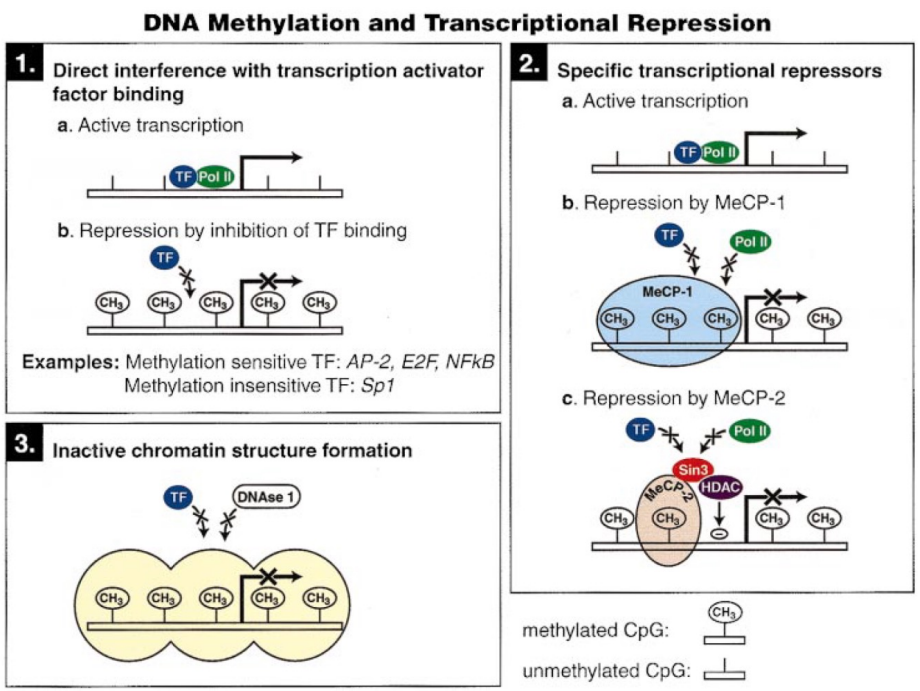


Electrophoretic mobility shift assay of AP-1 protein binding to the consensus AP-1 site of the human NT/N promoter

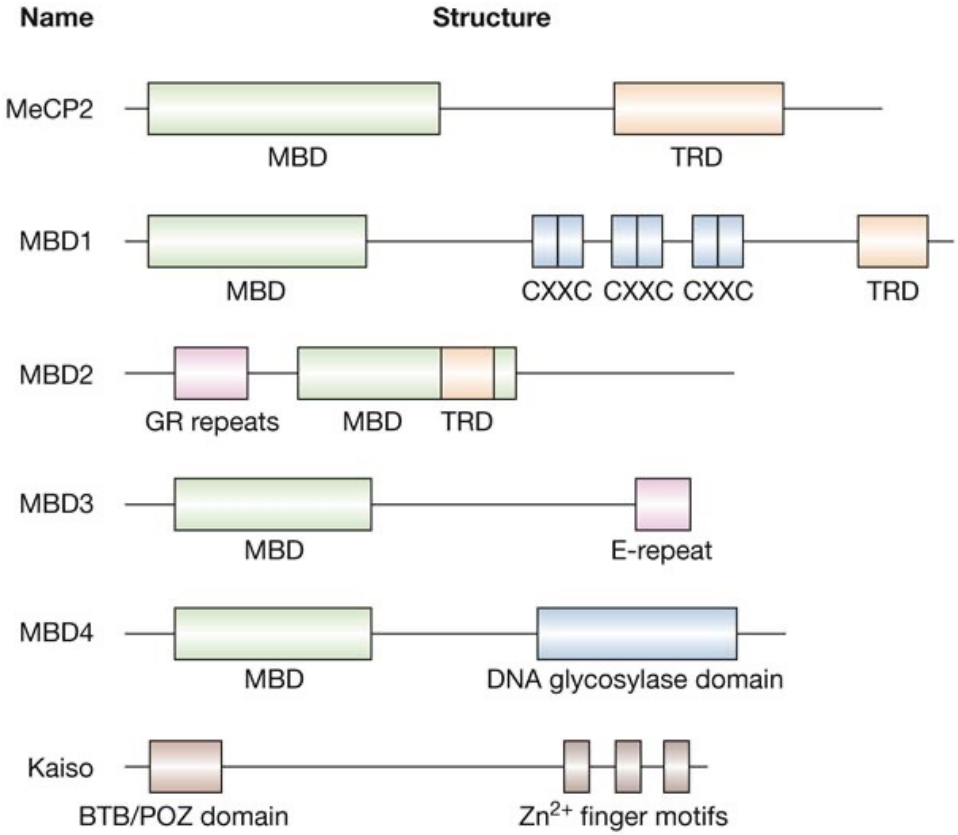


Dong Z et al. Am J Physiol Gastrointest Liver Physiol 2000;279:G1139-G1147

Mechanisms of DNA-methylation-mediated transcriptional repression

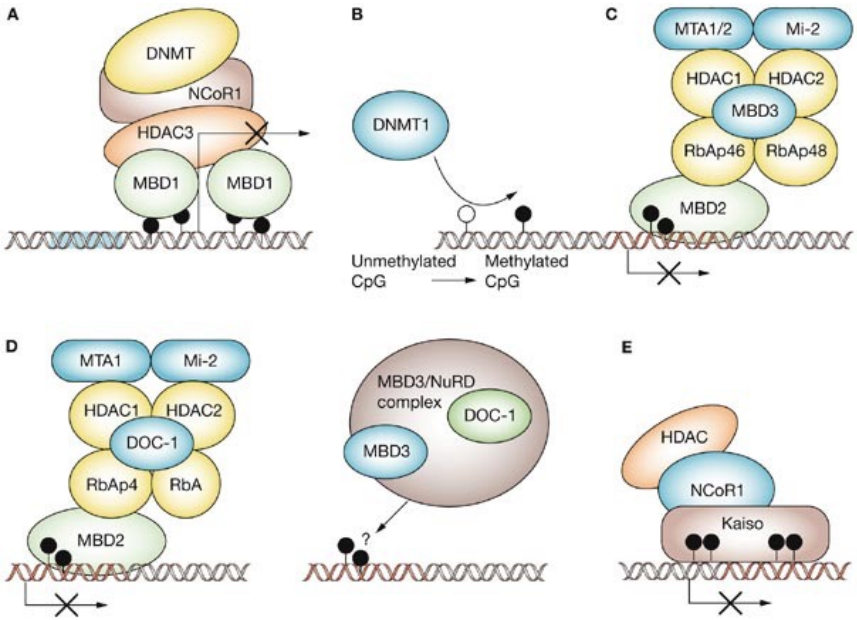


Methyl-binding proteins

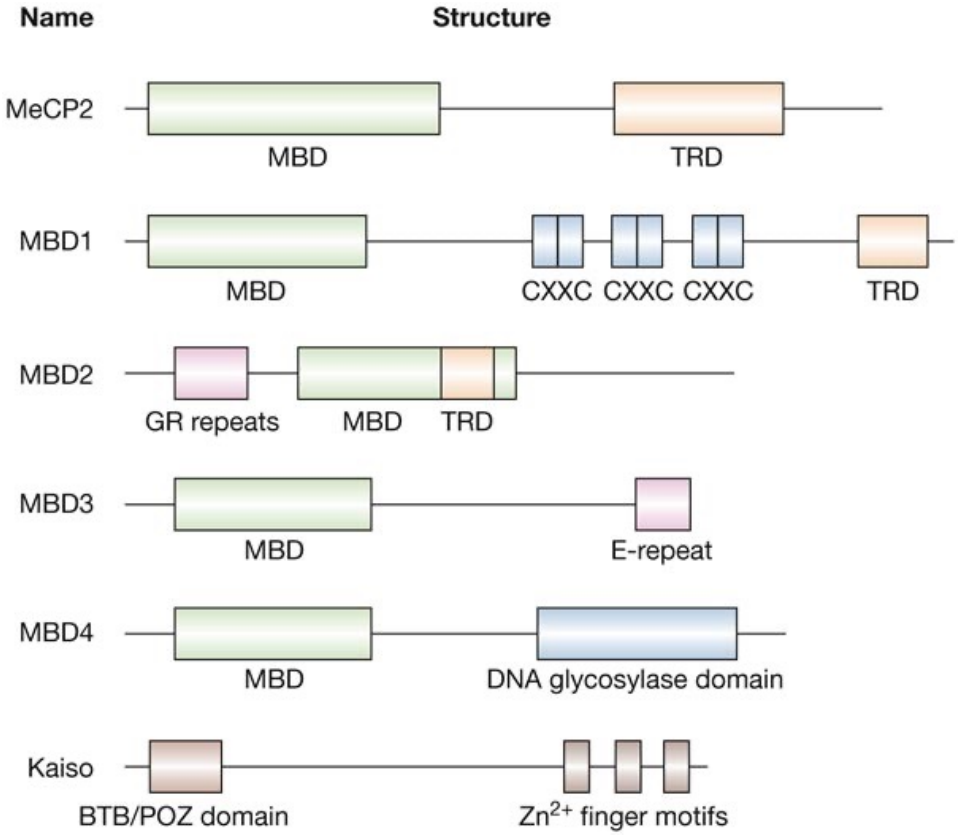


Mechanisms of DNA-methylation-mediated transcriptional repression

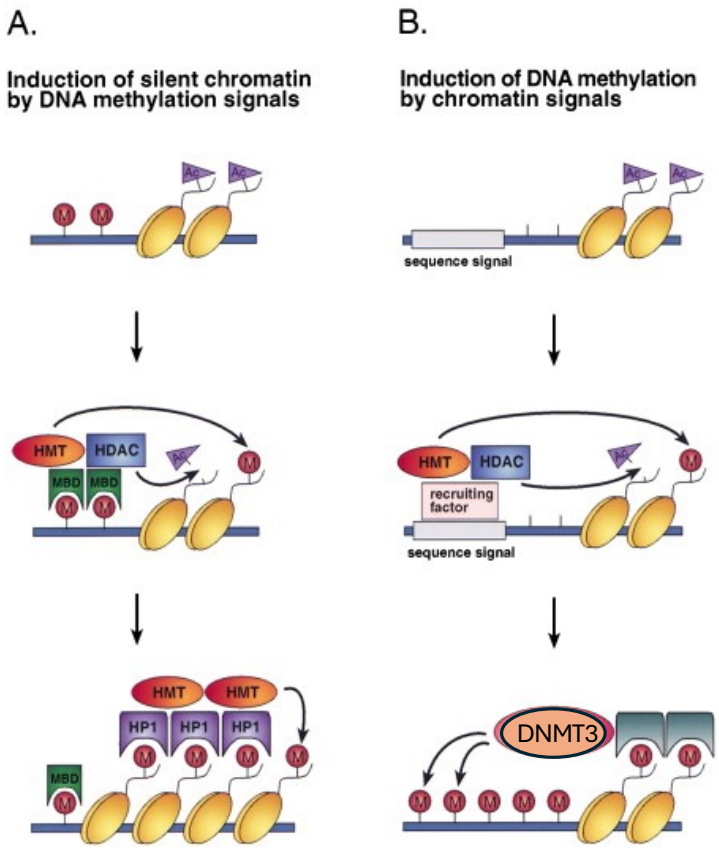
MBP recruit co-repressor complexes



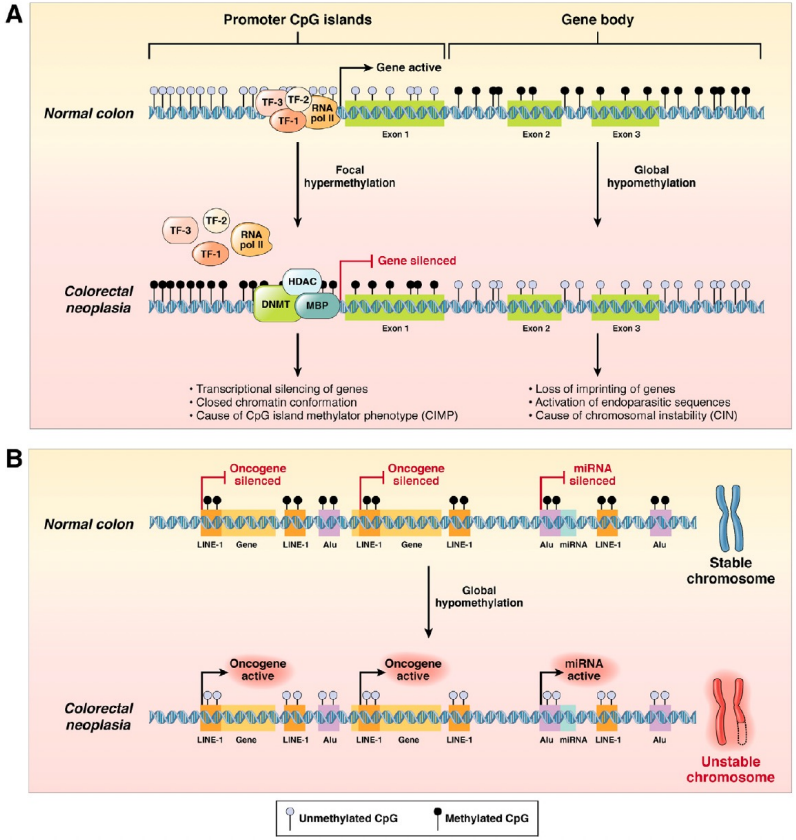
Methyl-binding proteins

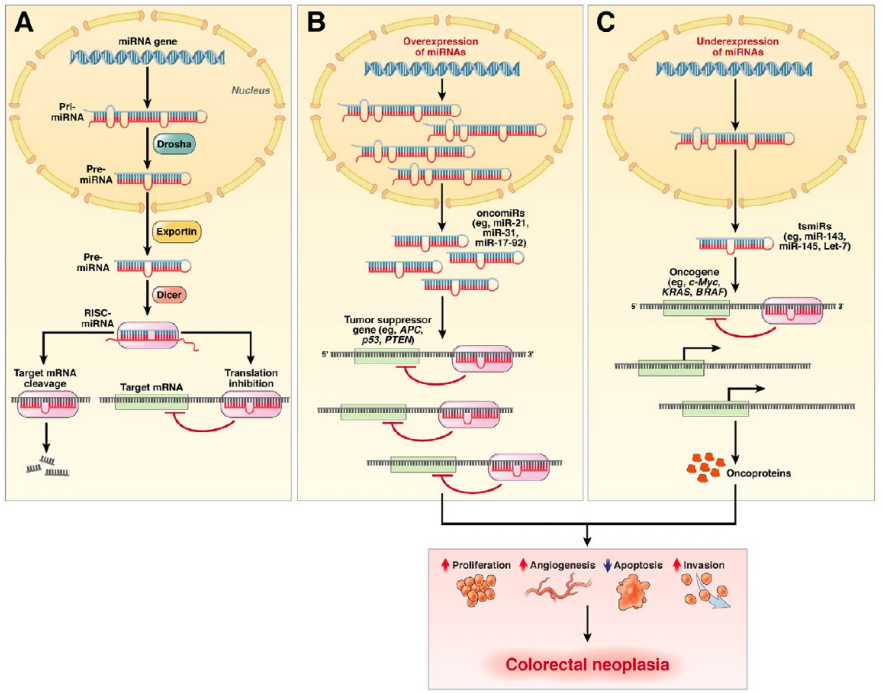


Mechanisms of DNA-methylation-mediated transcriptional repression



DNA-methylation in cancer





DNA-methylation in cancer

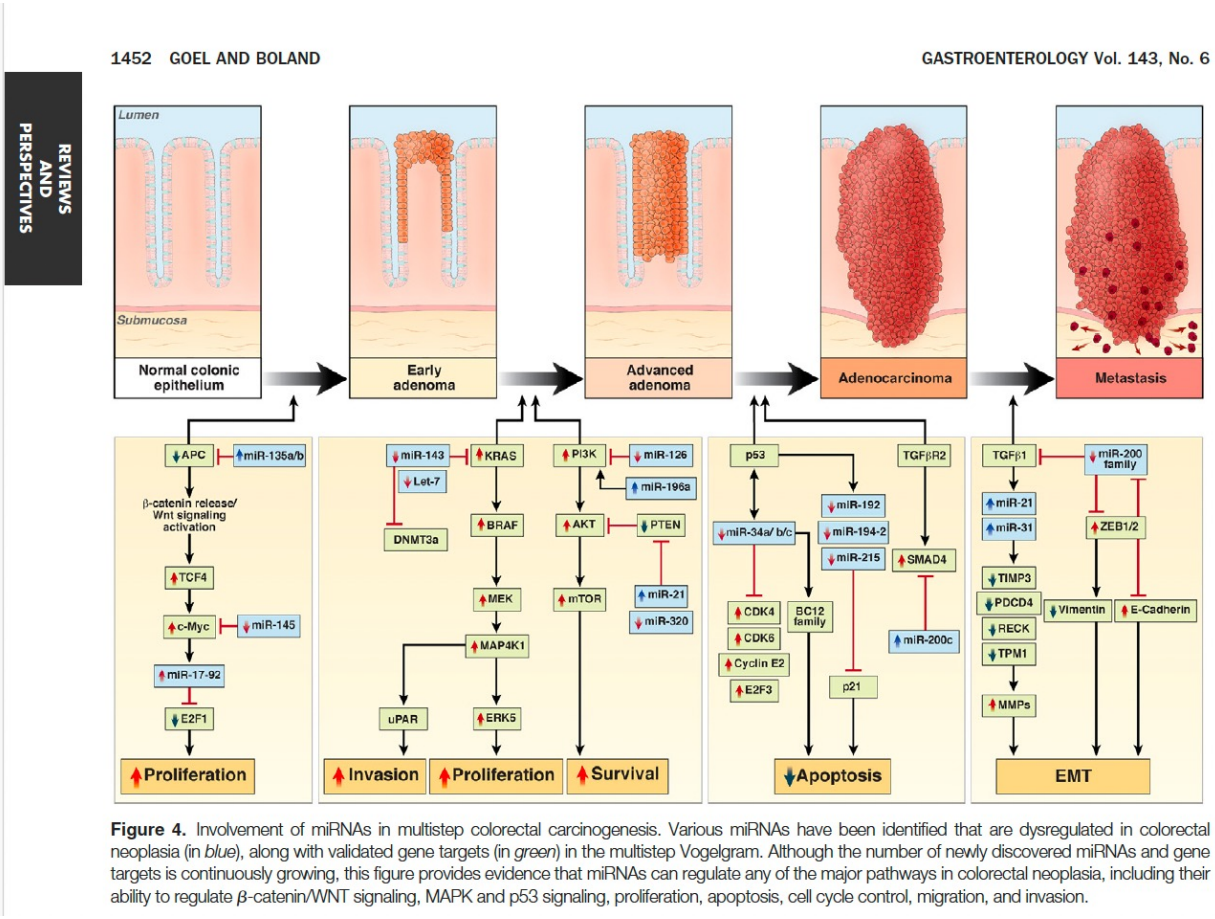


Figure 4. Involvement of miRNAs in multistep colorectal carcinogenesis. Various miRNAs have been identified that are dysregulated in colorectal neoplasia (in *blue*), along with validated gene targets (in *green*) in the multistep Vogelgram. Although the number of newly discovered miRNAs and gene targets is continuously growing, this figure provides evidence that miRNAs can regulate any of the major pathways in colorectal neoplasia, including their ability to regulate β-catenin/WNT signaling, MAPK and p53 signaling, proliferation, apoptosis, cell cycle control, migration, and invasion.

DNA-methylation in cancer

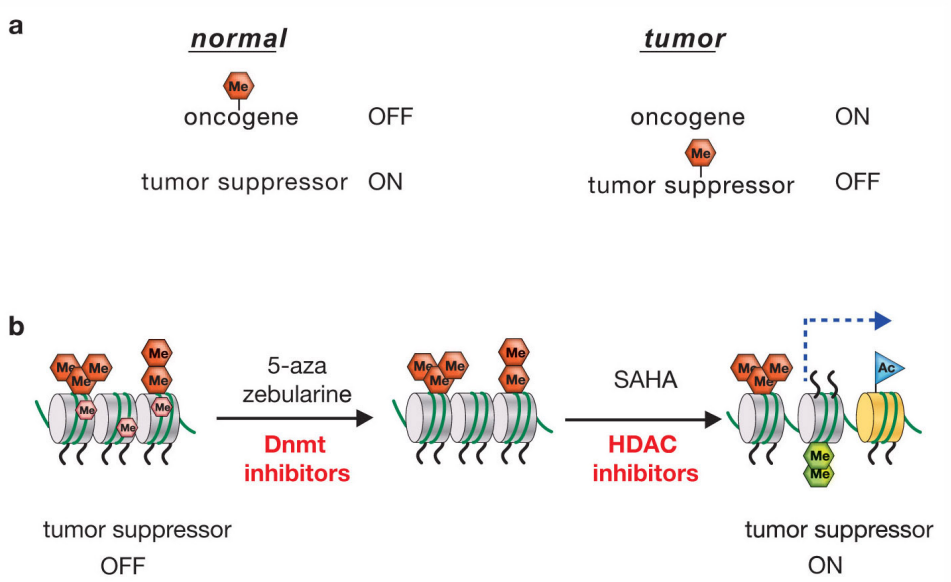


Figure 19. Epigenetic Modifications in Cancer

(*a*) Aberrant epigenetic marks at cancer-causing loci typically involve the derepression of oncogenes or silencing of tumor suppressor genes. Epigenetic marks known to alter a normal cell include DNA methylation, repressive histone methylation, and histone deacetylation. (*b*) The use of epigenetic therapeutic agents for the treatment of cancer has consequences on the chromatin template, illustrated for a tumor suppressor locus. Exposure to Dnmt inhibitors results in a loss of DNA methylation, and exposure to HDAC inhibitors results in the acquisition of histone acetyl marks and subsequent downstream modifications, including active histone methyl marks and the incorporation of histone variants. The cumulative chromatin changes lead to gene re-expression.

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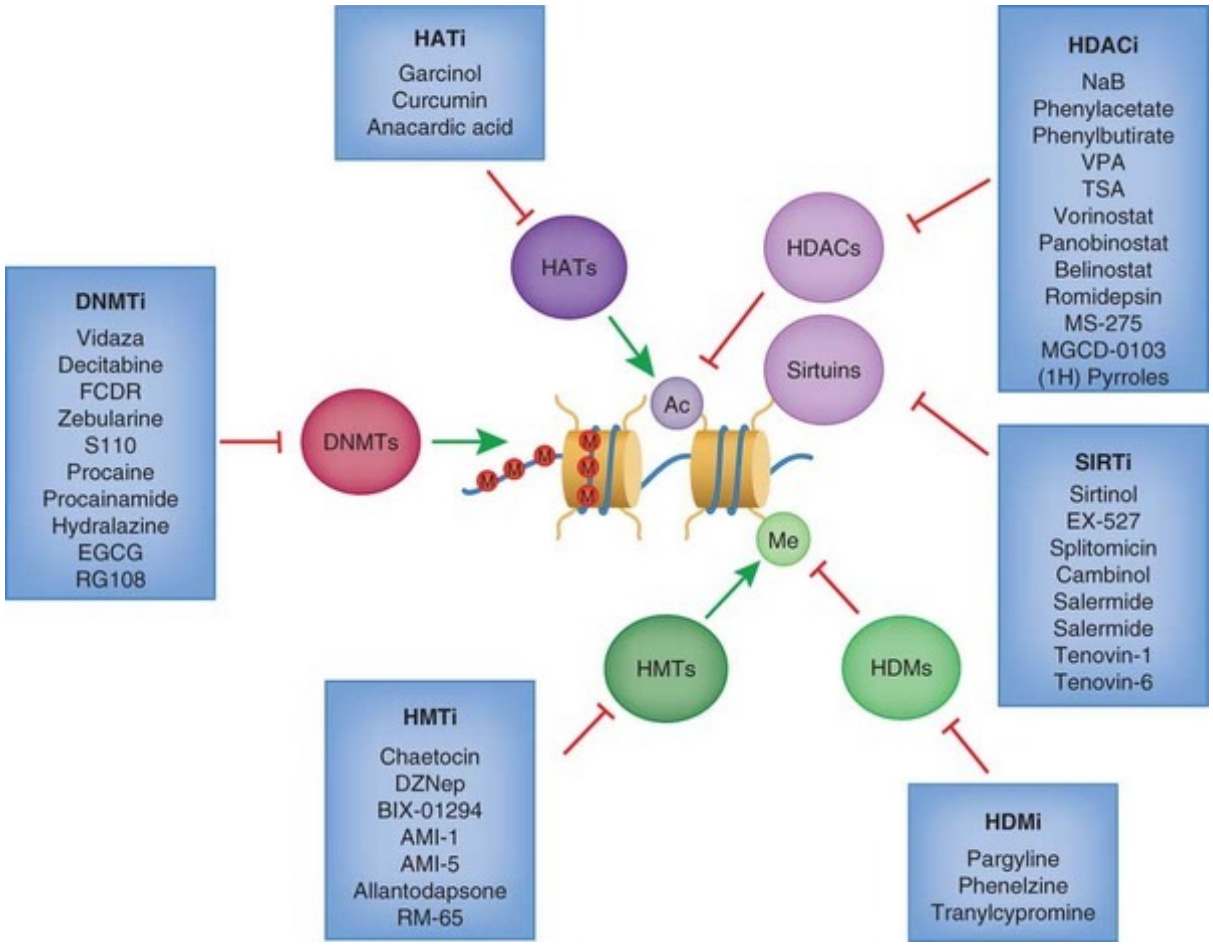
Epigenetic drugs